Tasmanian Institute of Agriculture

Effect of soil moisture availability and nitrogen source on nitrogen-use efficiency in cereals

By

Eseeri Kisaakye

BSc. Agric. (Hons), (Makerere University, Kampala, Uganda); MSc. Crop Improvement,

(The University of Nottingham, United Kingdom)

Submitted in fulfilment of the requirements for the

Doctor of Philosophy (Agriculture)

October, 2017

Declarations

This thesis contains no material which has been accepted for a degree or diploma by the University or any other institution and to the best of my knowledge contains no material previously published or written by any other person, except where duly acknowledged in the thesis.

Signature:

Eseeri Kisaakye

University of Tasmania

October, 2017

Authority of Access

This thesis may be made available for loan and limited copying and communication in accordance with the Copyright Act 1968.

Signature:

Eseeri Kisaakye

University of Tasmania

October, 2017

Acknowledgements

My sincere gratitude goes to Associate Professor Tina Acuña, my Primary Supervisor, for her guidance, knowledge, support and friendship, which she humbly and patiently gave me throughout the course of my PhD research. I thank my Co-Supervisor, Dr Peter Johnson, for his expertise, guidance and advice during my field experiments. Appreciation also goes to my Co-Supervisor, Professor Sergey Shabala for his knowledge and guidance during my laboratory studies and trusted level of efficiency, which was both reassuring and inspiring.

I greatly appreciate the University of Tasmania for offering me the tuition fee scholarship and the Grains Research and Development Corporation (GRDC) for the research funds provided. To Phil Andrews of the Horticultural Research Centre, Tasmanian Institute of Agriculture (TIA), University of Tasmania (UTAS), Brett Davey, Rob Howard and Rebecca Fish of the Tasmanian Institute of Agriculture, Mount Pleasant, Dr Lana Shabala of the Plant Physiology Laboratory, TIA, UTAS, Dr Alieta Eyles of the ARC Training Centre for Innovative Horticultural Products, UTAS and Dr Cai Shengguan of Western Sydney University, I am very grateful for the technical support provided. To Drs John Roger Otto and Dinah Borus, many thanks for the unwavering friendship and support.

I am grateful to the Cressy Research and Demonstration Station, for allowing me to carry out my field experiments at their Burlington Road site. I also acknowledge AgVita Analytical in Devonport, Tasmania for analysing the soil samples and the Central Science Laboratory (CSL) of the University of Tasmania for analysing the plant samples for nitrogen concentration.

Special gratitude goes to my parents (Godfrey Kaaya and Justine Nakitto Kaaya) and siblings (Mary Buttibwa, Joseph Ssempebwa, Milly Mirembe, Victoria Musasizi, Justine Mwesigwa, John Mutegesi, Ruth Muwanguzi and Margaret Kuteesa), for their continuous encouragement and support during my studies.

Finally, and most importantly, I would like to thank my husband (James Elvis Mbiru), daughter (Isabel Faith Kisakye), for their patience, encouragement and love.

Abstract

Cereal nitrogen-use efficiency (NUE) in developed and developing countries stands at 42 and 29% respectively. In Australia wheat NUE stands at 41% with the remaining 59% potentially contributing to environmental and health concerns. Processes such as ammonia volatilization, nitrification, leaching, erosion, runoff and denitrification account for 92% of nitrogen (N) loss from the plant-soil system. Achieving synchrony between N supply and crop demand is key to optimizing trade-offs among yield, profit and environmental protection in both large- and small-scale farming systems. Using enhanced-efficiency fertilisers such as controlled-release fertilisers (CRFs) may be one option to obtain a marked improvement in NUE in cereals. While soil moisture plays a significant role in plant growth and development, excessive soil moisture can cause significant yield losses. Waterlogging is a major abiotic constraint to cereal production in the high rainfall zones of Australia, particularly in areas with duplex soils. Understanding how we can improve cereal yield and NUE particularly under waterlogged conditions could help increase crop productivity.

This research sought to address three key research questions namely: (a) can timing of N application and source of applied N alleviate the adverse effects of waterlogging on wheat and barley growth and physiology; (b) can CRFs improve wheat yield, NUE and their components under waterlogged conditions; and (c) is the uptake of NH_4^+ along the root axis of selected wheat and barley varieties under hypoxia significantly higher than NO_3^-? These questions were answered through studies under glasshouse and field conditions as well as laboratory experiments.

Under glasshouse conditions, identified waterlogging sensitive wheat (cv. Mackellar) and barley (cv. Naso Nijo) varieties treated with conventional urea and a CRF were

waterlogged for 35 days and allowed to recover for another 35 days. During the study, leaf chlorophyll content and chlorophyll fluorescence were recorded on a weekly basis. Tiller number, green leaf area, above-ground dry matter (AGDM) and root DM were determined after waterlogging and recovery. In the field, the experiment was designed as a split-plot with irrigation regime and N fertiliser application as main-plot and subplot factors with three replicates. The irrigation regime included: rainfed, irrigated and waterlogged while N fertiliser application had nil N, single-applied urea, split-applied urea and CRF treatments. Nitrogen fertiliser was applied at a rate of 90 kg N/ha and piezometers were randomly installed to monitor the depth of the water table. Wheat growth and yield attributes including leaf area, leaf area index (LAI), tiller number, ear number, grain yield, AGDM, thousand grain weight (TGW) and harvest index (HI) were determined at specific growth stages namely: stem elongation (GS32), anthesis (GS61) and maturity (GS92). NUE, its components: nitrogen uptake efficiency (NupE) and nitrogen utilisation efficiency (NutE), N harvest index (NHI) and grain protein content (GPC) were also determined. Mapping the uptake of NH_4^+, NO_3^- and H^+ under hypoxia involved measurement of steady-state net fluxes of NH_4^+, NO_3^- and H^+ ions of wheat cv. Revenue and barley cv. Naso Nijo main roots at different positions along the root axis using non-invasive microelectrode ion flux estimation (MIFE) technique.

Under glasshouse conditions, the results showed that N fertiliser application improves leaf chlorophyll content, tiller number, green leaf area, AGDM and root DM during waterlogging and recovery. Differences between N treatments were evident with the CRF having the highest amount of AGDM for both selected wheat and barley varieties. Conventional urea on the other hand, improved wheat and barley growth at the start of waterlogging and significantly during recovery as the remaining urea was top-dressed. Under field conditions, the study findings showed a significant interaction between

irrigation regime and N fertiliser application for tiller number ($P = 0.013$) and leaf area ($P = 0.014$) at GS32. The AGDM per plant was significantly affected by irrigation regime ($P = 0.001$) and N fertiliser application ($P = 0.012$) while AGDM per unit area was significantly affected by the irrigation regime only ($P = 0.001$). At GS61, the irrigation regime ($P = 0.001$) and N fertiliser application ($P < 0.05$) had a significant effect on the tiller number, ear number and AGDM per plant. There was no significant interaction between the irrigation regime and N fertiliser application ($P > 0.05$). At maturity, yield attributes including tiller number, ear number and grain yield were significantly affected by irrigation regime ($P = 0.001$) and N fertiliser application ($P < 0.05$). The CRF had the highest grain yield for all irrigation regimes with 9.2 t/ha, 9.4 t/ha and 6.8 t/ha for the rainfed, irrigated and waterlogged, respectively. The urea treatments had an average of 8 t/ha for both the rainfed and irrigated, and 5.3 t/ha for the waterlogged. NUE was significantly affected by irrigation regime ($P = 0.001$) and N fertiliser application ($P = 0.036$). The waterlogged plants had the lowest NUE for all corresponding N treatments under the rainfed and irrigated regimes. The CRF had the highest NUE for all irrigation regimes. Under waterlogged conditions, the CRF improved NUE by 17% and 27% more than single- and split- applied urea, respectively. No significant variations were observed between N treatments for components of NUE, NHI or GPC for the rainfed and waterlogged treatments. Laboratory studies showed that the uptake and efflux of NH_4^+ and NO_3^- ions in wheat and barley varied significantly ($P = 0.001$) between different positions along the root axis at 20 µM and 1 mM NH_4NO_3 during normoxia and hypoxia. Hypoxia increased the uptake of NH_4^+ and NO_3^- for both wheat and barley and there was a preference for NH_4^+ over NO_3^-. Genotypic differences between wheat and barley were evident, with wheat having the highest uptake of both NH_4^+ and NO_3^- for the control and hypoxia at 20 µM and 1 mM.

It was concluded that N fertiliser application could alleviate the adverse effects of waterlogging in cereals. Applying full amount of the required fertiliser at sowing helps plants to withstand the adverse effects of transient and intermittent waterlogging through enhanced vegetative growth. Top-dressing urea after waterlogging and the termination of waterlogging to allow recovery enhances plant growth and development. Using CRFs may improve cereal growth and NUE though there might be no significant yield advantage over conventional urea to warrant investment. The findings also highlight the dynamic process of N uptake during hypoxia, which is characterised by significant variations over relatively short distances and periods of time. Hypoxic conditions also seem to trigger mechanisms that mediate N uptake resulting in higher uptake rates.

Refereed Conference Papers

Kisaakye, E, Acuña, TB, Johnson, P & Shabala, S 2014, 'Effect of water availability and nitrogen source on wheat growth and nitrogen-use efficiency', in *Proceedings of the 8th Annual Graduate Research Conference, Sharing Excellence in Research (SEiR)*, 4th - 5th September, University of Tasmania, Sandy Bay, Australia.

Kisaakye, E, Acuña, TB, Johnson, P & Shabala, S 2015a, 'Can nitrogen fertilizer application alleviate the adverse effects of waterlogging in wheat?', in *Proceedings of the 9th International Wheat Conference*, 20th - 25th September, The Four Seasons Hotel, Sydney, Australia.

Kisaakye, E, Acuña, TB, Johnson, P & Shabala, S 2015b, 'Effect of water availability and nitrogen source on wheat growth and nitrogen-use efficiency', in *Proceedings of the 17th Australian Society of Agronomy Conference*, 20th - 24th September, Wrest Point Convention Centre, Hobart, Australia, pp. 1-4.

Kisaakye, E, Acuña, TB, Johnson, P & Shabala, S 2017, 'Improving wheat growth and nitrogen-use efficiency under waterlogged conditions', in *Proceedings of the 18th Australian Society of Agronomy Conference*, 24th - 28th September, Mercure Ballarat Hotel and Conference Centre, Ballarat, Victoria, Australia, pp. 1-4.

Table of Contents

List of Figures

List of Tables

List of Abbreviations

[ ]: Concentration

AGDM: Above Ground Dry Matter

AMT/MEP: Ammonium transporter/methylammonium permease

ANOVA: Analysis of Variance

ARC: Australian Research Council

ATP or ATPs: Adenosine Triphosphate

B: Boron

C: Carbon

C: N: Carbon: Nitrogen ratio

$Ca(OH)_2$: Calcium Hydroxide

Ca: Calcium

Ca: Mg: Calcium: Magnesium ratio

Ca: NO_3: Calcium: Nitrate ratio

$CaCl_2$: Calcium Chloride

$CaCO_3$: Calcium Carbonate

cHATS: Constitutive High Affinity Transport System

CEC: Cation Exchange Capacity

CECe/ECEC: Effective Cation Exchange Capacity

CRF: Controlled Release Fertiliser

CRFs: Controlled Release Fertilisers

CSL: Central Science Laboratory

$CO(NH_2)_2$: Urea

Cu: Copper

$CuSO_4.\ 5H_2O$: Hydrated Copper (II) Sulfate

cv.: Cultivar

DM: Dry Matter

DTPA: Pentetic acid or diethylenetriaminepentaacetic acid

EA: Elemental Analyser

EC: Electrical Conductivity

EEFs: Enhanced-Efficiency Fertilisers

ETS: Electron Transport System

FC: Field Capacity

Fe: Iron

GHG: Green House Gas

GOGAT: GS/glutamine-2-oxoglutarate aminotransferase

GPC: Grain Protein Content

GS: Growth Stage

GWP: Global Warming Potential

H^+: Proton

H_2S: Hydrogen Sulfide

H_3BO_3: Boric Acid

HATS: High Affinity Transport System

HI: Harvest Index

HRC: Horticultural Research Centre

HRZ: High Rainfall Zone

iHATS: Inducible High Affinity Transport System

Inc.: Incorporated

K: Potassium

K^+: Potassium ion

KCl: Potassium Chloride

K_m: Michaelis-Menten kinetics

LA: Leaf Area

LAI: Leaf Area Index

LATS: Low Affinity Transport System

LIX: Liquid Ion Exchanger

LSD: Least Significant Difference

Ltd: Limited

MCP: Mono-Calcium Phosphate

Meq: Milliequivalents

Mg: Magnesium

$MgSO_4$: Magnesium Sulphate

MIFE: Microelectrode Ion Flux Estimation

Mn: Manganese

mRNA: messenger Ribonucleic Acid

N: Nitrogen

N_2O: Nitrous Oxide

NH_4^+: Ammonium ion

NH_4Cl: Ammonium Chloride

NHI: Nitrogen Harvest Index

NiR: Nitrite Reductase

NO_3^-: Nitrate ion

NPF: Peptide Transporter

NR: Nitrate Reductase

NRT: Nitrate Transporter

NUE: Nitrogen Use Efficiency

NupE: Nitrogen Uptake Efficiency

NutE: Nitrogen Utilisation Efficiency

P: Phosphorous

P: Probability

PBI: Phosphorous Buffering Index

PGRs: Plant Growth Regulators

PMF: Proton Motive Force

PO_4^{3-}: Phosphate ion

PTY: Proprietary

PVC: Polyvinyl chloride

Q_{10}: Temperature Coefficient

RNA: Ribonucleic Acid

S: Sulphur

SNS: Soil Nitrogen Supply

SOM: Soil Organic Matter

SPAD: Soil and Plant Analyses Development

SR/CR: Slow-release/Controlled-release

TGW: Thousand Grain Weight

TIA: Tasmanian Institute of Agriculture

USA: United States of America

UTAS: University of Tasmania

Chapter One: General Introduction

1.1 Introduction

With the global population projected at 9 billion by 2050 (Beddington 2010), increasing food production to feed the increasing population equitably and sustainably (Morison et al. 2008) is one of the current debates (Hirel et al. 2007). Even so, the increasing population and food demand is expected to rise along with the production of mineral fertilisers (Lubkowski & Grzmil 2007), as increasing fertiliser demand drives supply (FAO 2008). Recent trends in fertiliser costs are increasing the cost of food production (Rane et al. 2011). So far the doubling of agricultural production worldwide over the past four decades has been associated with a 7-fold increase in the use of nitrogen (N) fertilisers (Hirel et al. 2007). In the long term, an estimated 50-70% increase in cereal grain production to feed the projected 9.3 billion people by 2050 is expected to increase N use by the same magnitude (Ladha et al. 2005). Moreover, in the near future, an anticipated 38% increase in global cereal demand by 2025 can only be met by a 30% increase in N use in cereals (Dobermann & Cassman 2005), which may not be possible with the current technology.

The global N consumption has now crossed 100 million tons (Mt) (Rane et al. 2011), while the worldwide use of N in agriculture has increased to about 80 million t yr^{-1} (Olfs et al. 2005), of which cereals account for about 55% (17.3% for wheat) (Heffer 2009). Australia accounted for 0.9% (858,000t) of the world's N consumption between 2006 and 2008, 30.7% (263,000t) of which was used in wheat production (Heffer 2009). This consumption was expected to grow by 1.9% from 2010 to 2014 (FAO 2010) increasing N fertiliser use in crop production.

Unfortunately, the global nitrogen-use efficiency (NUE) for cereals is ~33% with an unaccounted 67% representing a US$15.9 billion annual loss of N fertiliser (Raun & Johnson 1999). Cereal NUE in developed and developing countries stands at 42% and 29%, respectively (Raun & Johnson 1999). In Australia wheat NUE stands at 41% with the remaining 59% potentially contributing to environmental and health concerns (Chen et al. 2008). A 2.4-2.7-fold increase in N driven eutrophication of ground and surface waters has been predicted by 2050 should the conversion of ~10^9 hectares of natural ecosystems for agricultural production occur (Tilman et al. 2001). This eutrophication may cause unprecedented ecosystem simplification, loss of ecosystem services and species extinctions (Giles 2005; Mathers et al. 2007; Tilman et al. 2001). Human health concerns particularly methemoglobinemia (blue baby syndrome) have also been reported in addition to the emission of nitrous oxide (N_2O), a greenhouse gas (GHG) with a global warming potential (GWP) of ~310 (Ju et al. 2009; Lubkowski & Grzmil 2007; Shaviv & Mikkelsen 1993). Nitrogen use in agriculture produces ~20% of the annual global reactive N (Dobermann & Cassman 2005). In Australia, agriculture is among the industries contributing to excessive loading of N into rivers particularly those in the high rainfall zones (HRZs) (Mathers et al. 2007) and the main source of N_2O. For instance, in 2005 agriculture contributed 67 Gg of N_2O of which N fertilisers accounted for 21 Gg (Chen et al. 2008). Processes such as ammonia volatilization, nitrification, leaching, erosion, runoff and denitrification account for 92% of N loss from the plant-soil system (Chen et al. 2008; Lubkowski & Grzmil 2007).

Arguably N fertiliser use, as is irrigation and use of improved seeds or crop varieties with desirable traits, is a key input to crop production improvement (Sinclair & Rufty 2012). However, the finite resources used in its manufacture (Haber-Bosch process) (Hirel et al. 2007) are dwindling consequently increasing their cost (FAO 2008; Stewart et al. 2008).

For example, more than US$45 billion per annum are spent on N fertilisers worldwide (Ladha et al. 2005) and this annual expenditure is expected to rise in future (FAO 2008).

With increasing costs and environmental pressures, improving NUE rather than higher fertiliser application rates is necessary to improve crop yields (FAO 2008). Improving NUE of crop plants through a combination of genetic improvement and agronomic approaches has been viewed as a holistic approach (Rane et al. 2011). Achieving a synchrony between N supply and crop demand is the key to optimizing trade-offs among yield, profit and environmental protection in both large-scale agricultural systems in developed countries and small-scale systems in developing countries (Cassman et al. 2002; Li et al. 2007; Rane et al. 2011).

Using enhanced-efficiency fertilisers such as slow/controlled-release (SR/CR) fertilisers (Li et al. 2007) may be one option to obtain a marked improvement in NUE (Chen et al. 2008) through synchronisation between N release and crop demand. Some studies have supported this notion as SR/CR fertilisers are said to reduce N loss thereby increasing N assimilation to more than 50% (Hauck 1985; Lubkowski & Grzmil 2007; Shaviv & Mikkelsen 1993). Other merits associated with SR/CR fertilisers include reduced anthropogenic eutrophication (Lubkowski & Grzmil 2007; Noellsch et al. 2009), and significant savings in labour, time and energy as single fertiliser application at sowing is possible precluding the need for topdressing (Lubkowski & Grzmil 2007; Mathers et al. 2007). Improving NUE will foster the tripartite goals of food security, agricultural profitability and environmental quality (Cassman et al. 2002; Mohammed et al. 2013).

Soil moisture also plays a significant role in plant growth and development; however, excessive soil moisture availability can cause significant yield losses (Zhang et al. 2006). Waterlogging is a major abiotic constraint to wheat production in the high rainfall zones

(HRZs) of Australia (Acuña et al. 2011; Bakker et al. 2005; Bakker et al. 2007; Zhang et al. 2006), particularly in regions with duplex soils (Yaduvanshi et al. 2012). Waterlogging is also increasingly becoming a matter of worldwide concern affecting 16% of soils in the USA and irrigated crop production areas of India, Pakistan and China (Colmer & Greenway 2011; Pang et al. 2004; Sairam et al. 2008). Waterlogging affects ca. 10% of the global land area (Setter & Waters 2003), with a yield penalty of 15-80% depending on the crop species, soil type and duration of the stress (Zhou 2010). On a global scale, about 10-15 million ha of the world's wheat growing areas are affected annually by waterlogging representing 15-20% of the 70 million ha cultivated annually for wheat production (Hossain & Uddin 2011; Yaduvanshi et al. 2012). In Australia, waterlogging causes an estimated annual production loss of AU$180 million (Pang et al. 2004). A greater proportion of this is incurred in Western Australia where it reduces spring wheat yield by ~30-55% (Zhang et al. 2006), causing AU$100 million in crop losses in a wet year (Zhang et al. 2004). In Tasmania, the relatively high rainfall coupled with a range of soil types, leads to a high incidence of waterlogging in winter, causing yield losses of 39-49% in wheat despite its relative tolerance compared with barley (Zhou 2010).

Waterlogging reduces N net uptake and transport in plants (Malik et al. 2002). As soon as O_2 is depleted, the NO_3^- is used by microorganisms in the soil as an alternative electron acceptor in their respiration allowing the electron transport system (ETS) and oxidative phosphorylation to continue (Drew 1991). The NO_3^- is reduced to NH_4^+, which becomes the major form of mineral N in waterlogged soils (Colmer 2015; Feng et al. 2011; Yan et al. 2011). The increase in abundance of NH_4^+ is accompanied by about equimolar H^+ production. The protons excreted due to increased H^+-ATPase activity lead to acidification of the rhizosphere and repressed cation uptake (von Wirén et al. 2000). Nutrient uptake by plants can be severely reduced under anaerobic or hypoxic conditions created during

waterlogging. Understanding the transport of mineral nutrients such as NH_4^+ and NO_3^- within the plant can shed light on the processes and mechanisms either up-regulated or down-regulated during hypoxia (Kiba & Krapp 2016). This will also give us more insight into how hypoxia affects plant N nutrition.

There are compelling reasons for increasing NUE in agricultural systems while maintaining or decreasing N inputs. Efficient N fertiliser management is critical for the economic production of cereals and the long-term protection of the environment (López-Bellido et al. 2006). New technologies employing SR/CR fertilisers can be used as effective mitigation alternatives to control environmental impacts of fertilisation (Shoji et al. 2001). The knowledge gained from this study will equip cereal producers with an opportunity to make informed decisions about SR/CR fertiliser use in cereal production rather than their predominant use in non-agricultural crops (Shaviv & Mikkelsen 1993). Through field experiments, studies under controlled glasshouse conditions and laboratory studies; this research addressed the following objectives.

1. To assess selected wheat and barley varieties for tolerance to prolonged waterlogging;
2. To determine the effect of waterlogging, nitrogen source and subsequent recovery on wheat and barley growth and physiology;
3. To investigate whether timing of N application and source of applied N can alleviate the adverse effects of waterlogging on wheat growth and improve NUE;
4. To characterise the uptake of NH_4^+, NO_3^- and H^+ ions along the root axis of selected wheat and barley varieties under hypoxia.

The hypotheses tested were:

(a) Waterlogging will significantly decrease the growth and development of all selected wheat and barley varieties;

(b) The controlled-release fertiliser (CRF) will significantly increase the growth and physiological responses of selected wheat and barley varieties than conventional split-applied urea;

(c) Under similar irrigation management, the CRF will significantly increase wheat growth attributes, yield components, NUE, NUE components, NHI and GPC compared with the single- and split-applied urea treatments;

(d) The uptake of NH_4^+, NO_3^- and H^+ will vary significantly along the root axis and between genotypes, and the uptake of NH_4^+ will be significantly higher than NO_3^-.

1.2 Thesis structure

The thesis contains seven chapters; a brief description of each chapter is given below.

Chapter 1: General Introduction: gives a brief insight into the study and provides a general overview of the thesis.

Chapter 2: Literature Review: gives a detailed review of this research highlighting the dynamics of N fertiliser use in cereal production and how NUE can be improved through the use of enhanced-efficiency fertilisers. It further shows how the duplex nature of soils in Tasmania impact on crop productivity through waterlogging. The review also provides a review of the kinetics and molecular mechanisms mediating uptake of N under hypoxia.

Chapter 3: includes two experiments undertaken under controlled conditions in pots. The first experiment assessed selected wheat and barley varieties for tolerance to prolonged

waterlogging. In this study, it was hypothesised that waterlogging will significantly decrease the growth and development of all selected wheat and barley varieties. The second study focused on the effect of waterlogging, nitrogen source and subsequent recovery on wheat and barley growth and physiology. The key objective was to assess the response of selected wheat and barley varieties to N fertiliser application with a hypothesis that the CRF will significantly increase the growth and physiological responses of selected wheat and barley varieties than conventional split-applied urea.

Chapter 4: includes a field study, which investigated whether timing of N application and source of applied N can alleviate the adverse effects of waterlogging on wheat growth and improve NUE. The study evaluated wheat growth responses and yield components under different irrigation regimes and nitrogen treatments and quantified NUE, its components and grain protein content under different irrigation regimes. It was hypothesised that under similar irrigation management, the CRF will significantly increase wheat growth attributes, yield components, NUE, NUE components, NHI and GPC compared with the single- and split-applied urea treatments.

Chapter 5: maps the uptake of NH_4^+, NO_3^- and H^+ ions along the root axis of selected wheat and barley varieties under hypoxia using the non-invasive microelectrode ion flux estimation (MIFE) technique. In this study, it was hypothesised that the uptake of NH_4^+, NO_3^- and H^+ will vary significantly along the root axis and between genotypes, and the uptake of NH_4^+ will be significantly higher than NO_3^-.

Chapter 6: gives a general discussion of the major findings of the study.

Chapter 7: gives a conclusion of the main results, recommendations and areas warranting further investigations.

Chapter Two: Literature Review

2.1 Introduction

Waterlogging affects ca. 10% of the global land area (Setter & Waters 2003), and is one of the most important abiotic constraints to agricultural crop production (Zhang et al. 2006). The yield penalty resulting from waterlogging may vary between 15% and 80% depending on the crop species, soil type and duration of the stress (Zhou 2010). Waterlogging reduces nutrient uptake (Malik et al. 2002) as the reduction in nutrient availability is enhanced by shoot growth relative to the roots, a phenomenon known as the 'dilution effect' (Colmer 2015). Nutrient uptake is also inhibited by lack of energy to drive the primary and secondary active transport via the H^+-translocating ATPase in the plasma membrane (Armstrong & Drew 2002).

The application of N fertilisers can improve plant growth and development under waterlogged conditions (Pang et al. 2007; Robertson et al. 2009; Swarup & Sharma 1993). In order to improve NUE and plant productivity under waterlogged conditions, slow-release/controlled-release (SR/CR) fertilisers could be a possible option. Understanding the pattern of N uptake along the root axis will illuminate the processes and mechanisms influencing N uptake during hypoxia.

This review highlights the dynamics of N fertiliser use in cereal production and how NUE can be improved through the use of enhanced-efficiency fertilisers. It further shows how the duplex nature of soils in Tasmania impacts on crop productivity through waterlogging and gives an insight into the kinetics and molecular mechanisms mediating uptake of N under hypoxia.

2.2 Tasmanian climate

The island of Tasmania lies between 144.5^0 and 148^0E and stretches 40^0 to 43.5^0S (Hill et al. 2009). It is characterised by a cool-temperate climate with variable annual rainfall ranging between 500 mm in the central Midlands and 1000 mm on the north-west coast (Acuña et al. 2011). Despite this variability in rainfall, Tasmania is located in the high rainfall zone (HRZ) of Australia. A HRZ is defined as an area receiving at least 550 mm of annual average rainfall (Mathers et al. 2007).

2.3 Cereal production in Tasmania

Cereal production in Tasmania accounts for 16,000 ha mainly dominated by wheat, barley and oats (ABARES 2017). In 2016-17, 34 and 17 kilotons of wheat and barley respectively were produced with only a slight increase of 2 kilotons for each from the previous year (ABARES 2017). Unlike barley, wheat production in the region has been expanding due to the high rainfall, longer and cooler growing seasons leading to higher yields (Mathers et al. 2007; Zhang et al. 2006). A typical wheat growing season is around 8.5 (May-January) and 10.5 months for grain and dual-purpose crops, respectively with an average yield of ~6-8 t ha^{-1} (Acuña et al. 2011). Most of Tasmania's cereals are used for animal feed with limited demand for milling wheats (GRDC 2011). Nonetheless, access to irrigation and high-yielding winter wheat varieties are expected to stimulate grain production (GRDC 2011).

2.4 Nitrogen dynamics

Crop production is by far the single largest cause of human alteration of the global N cycle (Liu et al. 2010). The supply of N is dependent on applied fertiliser, soil type, micro-environment around the root zone and the mechanisms of N loss into the environment (Figure 2.1). These factors are primarily influenced by the crop and fertiliser type, method

and amount applied and crop season. On the other hand, the primary determinants of total plant-available N supply are net rate of N release from the soil organic matter (SOM) and incorporated crop residues. These are controlled by the balance between N immobilization and mineralization as mediated by the soil microbes, contributions from applied organic and inorganic N sources and losses from the plant-available pool (Cassman et al. 2002). Other contributions include: wet and dry depositions from rainfall and dust, free living biological N fixation (BNF) and in irrigated systems, N contained in water (Cassman et al. 2002). Additionally, under temperate conditions a flush of N mineralization after thawing and warming in the spring season usually occurs, which boosts available N (Cassman et al. 2002).

Regardless of the N source, the potential for N loss often occurs when the size of plant-available N pool exceeds the crop uptake requirements (Cassman et al. 2002). Much of the concern about N fertilisers emanate from the fact that only a fraction of applied N is utilised by crop plants (Rane et al. 2011). Usually, N is predominantly lost in form of leaching and denitrification of nitrate-N (NO_3^-N) into N_2O; however, denitrification is a common occurrence on naturally heavy and poorly drained soils (Rane et al. 2011). Other mechanisms of N loss include: nitrification (Jarvis 1996), immobilization (Harper et al. 1987), volatilization of surface-applied urea in form of NH_3, N_2, NO and NO_2 and surface runoff particularly during heavy precipitation (Fillery & McInnes 1992; Mathers et al. 2007). Under temperate climate, NO_3^- leaching is exacerbated by the presence of negatively charged soils, which limit the presence of NO_3^- ions. In addition, the presence of NH_4^+ ions in the soil is limited as they are readily oxidized to NO_3^- ions by microorganisms (Di & Cameron 2002) particularly in aerated soils (Schenk 1996). Understanding N cycling in the soil-plant atmosphere components of cereal production systems is necessary to maximize yield and quality (Harper et al. 1987). In cereals, plant N

concentration peaks in the early vegetative phase and decreases during the remaining growth period even though N uptake from soil continues until maturity (Harper et al. 1987). This decrease has been attributed to the slower rate of N assimilation relative to C and large losses of N between anthesis and grain filling mainly in form of NH_3 (Harper et al. 1987). N is lost from the leaves during leaf photorespiration and due to the imbalance between N accumulation and N assimilation in plants (Xu et al. 2012). In addition, during grain filling, more N is transferred from the leaves to the grain compared with the stem, which boosts grain N content (Harper et al. 1987). On the other hand, application of N fertiliser near anthesis has been suggested to increase post-anthesis N uptake, grain protein content and grain protein concentration (Mohammed et al. 2013; Wuest & Cassman 1992b).

The efficiency of N assimilation depends on complex interactions in the crop's root system, microorganisms, chemical reactions taking place in the soil and processes leading to adverse elimination of N from the soil (Lubkowski & Grzmil 2007). We can improve the overall NUE of a cropping system by increasing crop uptake efficiency from applied N inputs and reducing the amount of N lost from soil organic and inorganic N pools (Cassman et al. 2002).

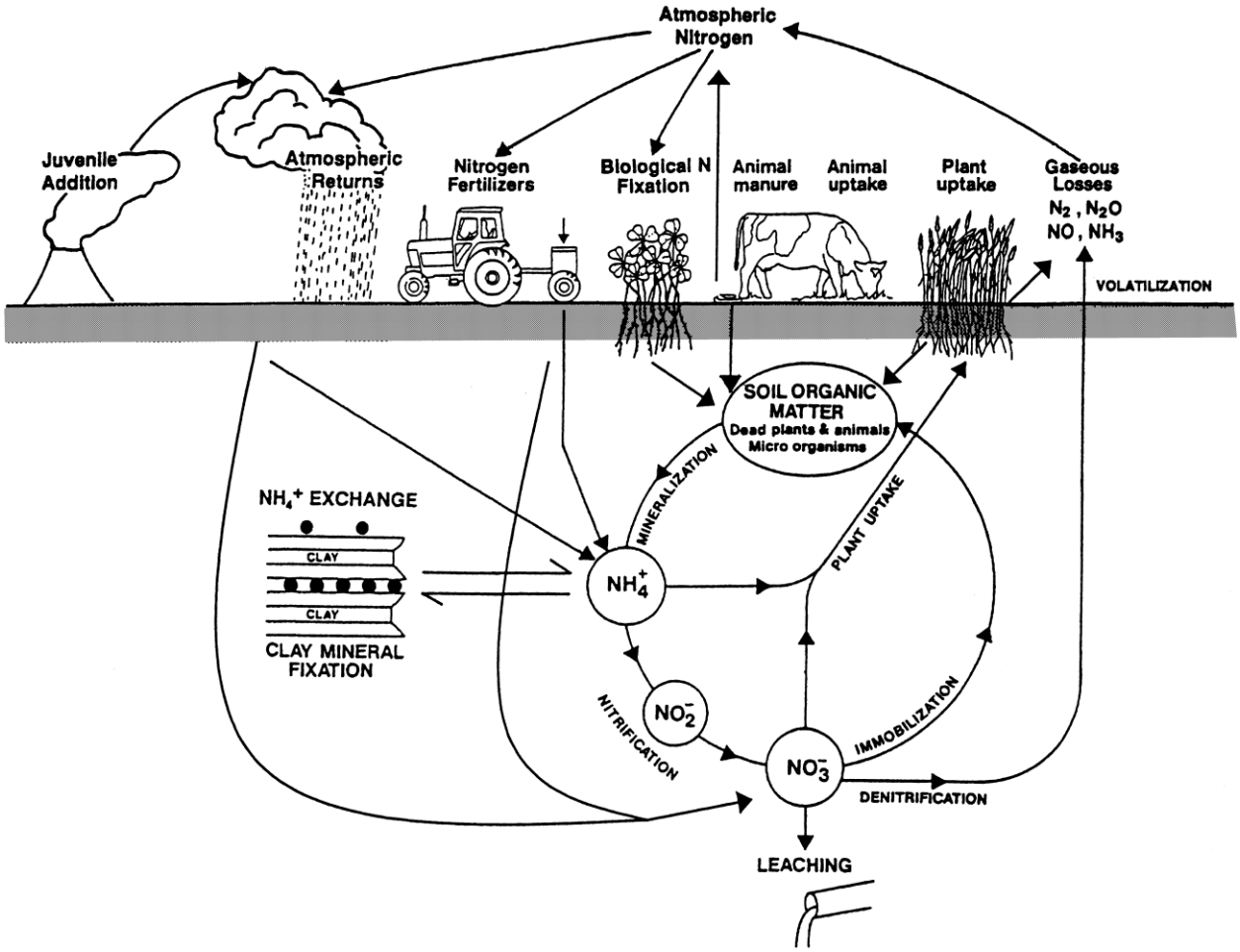

Figure 2.1: Nitrogen dynamics in the soil-plant system. Source: Di and Cameron (2002).

2.4.1 Role of nitrogen fertiliser in cereal production

In most commercial cropping systems, the natural supply of N is often insufficient to meet crop N demand, which necessitates inorganic N fertiliser application. The commonly available N fertilisers in Australia used in cereal broadacre cropping include urea, di-ammonium phosphate and mono-ammonium phosphate (Mathers et al. 2007). Nitrogen is mostly absorbed from the soil through mass transfer as NH_4^+ and NO_3^- ions by the crop's root system (Lea & Azevedo 2006), and is one of the most important nutrients responsible for large yields obtained from high input agriculture (Cai et al. 2011; Cai et al. 2012; Ladha et al. 2005).

Constituting 2 to 4% of the above-ground plant dry material (Mengel & Kirkby 2001), N is among the important macro-nutrients vital for plant growth. In the leaf, a significant proportion of the total N is allocated to the chloroplasts (Shangguan et al. 2000b), most of which is used for synthesis of components of the photosynthetic apparatus particularly ribulose-1,5-bisphosphate carboxylase/oxygenase, (RuBisCO), which plays a significant role in CO_2 assimilation (Hirel et al. 2007; Shangguan et al. 2000a; Shangguan et al. 2000b). There is a positive correlation between the photosynthetic capacity of leaves and their N content (Cai et al. 2011; Cai et al. 2012; Shangguan et al. 2000b). Previous research has shown that N fertiliser application promotes N uptake, which in turn increases chlorophyll content, leaf area index (LAI), photosynthetic rate and resultant crop yield (Wang et al. 2012). In cereals, N supply has a significant effect on vegetative growth particularly tillering and canopy size and duration, which influences resultant biomass, grain yield (Anderson 1985; Cai et al. 2012; Fischer 1993; Fois et al. 2009; Latiri-Souki et al. 1998), and grain protein content (Ercoli et al. 2008; Latiri-Souki et al. 1998).

2.4.2 Timing and application of nitrogen fertilisers

Nitrogen management is a vital aspect in improving NUE and maximizing crop productivity (Cai et al. 2012; López-Bellido et al. 2006). This aspect encompasses the mode-, timing- and rate- of N application (Kassem & Al-Suker 2009; Zhang et al. 2006). Methods of N application include: deep placement, split-application and fertigation (Chen et al. 2008). Other methods of N application include banding and broadcasting (Gordon 2009; Kassem & Al-Suker 2009). However, care should be taken during banding and deep placement as N placed in contact with the seed may cause crop injury (Gordon 2009). A 3-4 cm distance from the plant root system is often recommended (Lubkowski & Grzmil 2007).

Usually, under split application, a smaller proportion of N is applied at planting or pre-planting as a basal and the remaining N, often the largest proportion is top dressed at tillering or stem elongation or even at near anthesis (Mohammed et al. 2013). This strategy avoids unnecessary vegetative growth and risk of lodging (Anderson 1985). A study by Wang et al. (2012) showed that a ratio of 3:7 for basal fertiliser to top dressed N can give promising results such as high biomass, grain yield and improved grain quality. The basal fertiliser enhances nutrient uptake from the soil (Gordon 2009), through stimulation of early vegetative growth (Zhang et al. 2006; Pang et al. 2014), producing high tiller number per m^2 (Sieling et al. 1998). However, high N amounts at planting may reduce NUE and the N harvest index (NHI) as additional ears derived from higher shoot categories yield less than the main ears (Sieling et al. 1998). The reduction in yield could be due to increased competition for other nutrients or water, which induces water stress during grain filling (Zhang et al. 2006) causing 'haying off' in wheat (van Herwaarden et al. 1998) and terminal drought (Farooq et al. 2014).

Besides that, excessive application of N fertiliser at planting increases the risk of NO_3^-N leaching and immobilization (Mohammed et al. 2013), thereby reducing NUE without any increase in yield (Li, X et al. 2007). This is supported by Wuest and Cassman (1992a) and López-Bellido et al. (2006), who observed a 30-55% recovery of N applied at planting compared with 55-80% recovery of N applied at anthesis. Similarly, López-Bellido et al. (2005) obtained a mean wheat use of N fertiliser of 14% and 55% when N was applied at sowing and top-dressed during stem elongation, respectively. Turning to Freney et al. (1992), post sowing N application yielded 50-70% N recovery in comparison to >40% for N applied at sowing. Studies in the United Kingdom have also showed that spring N application could lead to 50-80% crop N recovery as this season is synonymous with tillering and faster growth (Di & Cameron 2002). Late N application between booting and

anthesis has been suggested to increase grain yield and N content particularly on poorly drained soils (Mascagni Jr & Sabbe 1991).

However, contrary to the above, a few studies have argued that similar quantities of N can be assimilated despite whether the entire N is applied at planting or split applied between planting and flowering (Strong 1982). Krishnakumari et al. (2000) concluded that split application where 50% of the total N is applied at tillering and flowering respectively can increase N and grain protein content without incurring any yield penalty. Much as late N application may improve NUE, applications after stem elongation have been claimed to affect N utilization efficiency (NutE) (Fischer 1993). Furthermore, the traditional strategy of split N at application has been criticized for producing more straw, which results in a low grain to straw ratio (Krishnakumari et al. 2000). While the adequacy of a single N application throughout the duration of the growing season is dependent on the quantity applied and subsequent growth conditions (Strong 1982).

Arguably, numerous studies have been conducted to evaluate the effect of timing of N fertiliser application on wheat yield and to a larger extent N recovery using ^{15}N-labelled isotope (Belford et al. 1992; Di & Cameron 2002; Freney et al. 1992; López-Bellido et al. 2006; López-Bellido et al. 2005). However, using ^{15}N-labelled isotope doesn't thoroughly evaluate NUE, NutE and NupE. In addition, while split N fertiliser application is a highly favoured strategy, it is associated with labour costs, challenges with fertiliser placement and application after crop establishment can cause mechanical and scorch damage such as foliar burn (Oertli 1980; Phillips & Mullins 2004). One such strategy to overcome the challenges associated with single- and split- application of conventional fertilisers particularly urea is the use of enhanced-efficiency fertilisers such as slow/controlled-release (SR/CR) fertilisers.

As plant nutritional needs vary considerably during individual growth stages, pragmatic measures are needed to maintain ample nutrient supply during critical growth stages (Anderson 1985; Oertli 1980). Nitrogen being a vital nutrient for plant growth, appropriate N management should be ensured to maximize NUE and yield attributes in cereals (Anderson 1985; Oertli 1980). Furthermore, given the complexity of the N cycle, spatial variability in soils and continued release of improved varieties with higher N demand, correct timing of N application remains important (Mohammed et al. 2013). This could be achieved by synchronizing N supply with crop demand, a mechanism which has proved possible with SR/CR fertilisers.

2.4.3 Use of slow/controlled-release fertilisers in cereal production

Inorganic N is needed to supplement soil N supply (SNS) (Freney et al. 1992), however, the available conventional N sources are easily lost through leaching (NO_3^-), denitrification (N_2, N_20), immobilization, volatilization (NH_3) and surface runoff (Ju et al. 2009; Lubkowski & Grzmil 2007; Mathers et al. 2007; Shaviv & Mikkelsen 1993). Consequently, advances in fertiliser development have increased to improve NUE leading to the design of SR/CR fertilisers (Rane et al. 2011; Shaviv 2001; Trenkel 1997; Trenkel 2010). The world consumption of SR/CR fertilisers was estimated at 786,000 metric tonnes in 2004/05, a 45% increase compared with 1995/96 (Trenkel 2010). The global demand for CRFs is expected to increase at around 10% annually during 2014-19 (IHS Markit 2015). While their market value was estimated at USD 2.2 billion in 2014, it is projected to reach USD 3.2 billion by 2020 (Markets & Markets 2017) and exceed USD 3.9 billion by 2024 (Global Market Insights Inc. 2017).

According to Shaviv and Mikkelsen (1993), SR/CR fertilisers are defined as those from which nutrient release into the environment occurs in a more or less controlled manner.

They are generally classified into three main types: (a) inorganic low solubility compounds; (b) organic low solubility compounds; and, (c) coated materials in which a physical barrier controls their release (Chen et al. 2008; Shaviv 2001). Mechanisms of nutrient release by SR/CR fertilisers include: diffusion, osmotic pumping and convective release by coating disruption (Adams et al. 2013). These mechanisms have been extensively discussed by Hauck (1985); Lubkowski & Grzmil (2007); Shaviv (2001); Trenkel (2010). Nutrient release from SR/CR fertilisers is largely dependent on temperature (Adams et al. 2013), with a 10^0C rise reported to double nutrient release (Huett & Gogel 2000; Kochba et al. 1990). However, soil moisture content plays a significant role in nutrient release (Wang & Alva 1996).

Nutrient release by CRFs is influenced by soil moisture content (Gandeza & Shoji 1992; Wang & Alva 1996). According to Kochba et al. (1990), the rate of nutrient release from the CRF is determined by the migration of vapour from the soil into the fertiliser granule. However, this is dependent on the fertiliser nutrient release mechanism (Kochba et al. 1990). Fertilisers whose nutrient release is largely dependent on soil temperature, moisture content of 50-100% FC has little effect on nutrient release rate (Huett & Gogel 2000; Kochba et al. 1990). But at 25% FC, nutrient release from SR/CR N fertilisers is usually slower and completely stops in dry soils (Kochba et al. 1990). This is attributed to the marked effect of low soil moisture content on the diffussion process and mass movement of water molecules in the soil (Hillel 1980; Kochba et al. 1990). On the other hand, excessive soil moisture due to waterlogging or flooding can cause significant N losses from readily soluble fertiliser forms such as conventional urea through leaching (Wang & Alva 1996), unlike CRFs, which have been formulated to minimise the leaching loss of applied N (Alva 1992; Hauck 1985). Nonetheless, nutrient loss through leaching is

dependent on soil type and the intensity and duration of waterlogging (Wang & Alva 1996).

Often referred to as enhanced-efficiency fertilisers (Chen et al. 2008), SR/CR fertilisers are designed to release N over an extended period of time during crop growth (Lubkowski & Grzmil 2007; Shaviv & Mikkelsen 1993; Trenkel 2010). They maximize NUE by synchronizing N release with crop demand (Shaviv & Mikkelsen 1993; Trenkel 2010). Some of the commercially available SR/CR fertilisers in Australia include: polymer-coated urea, polyolefin-coated urea and humic-acid-coated urea (Chen et al. 2008).

As mentioned earlier, SR/CR N fertilisers are viewed as a possible option to improve NUE. Some studies have supported this notion as SR/CR fertilisers are said to reduce N loss thereby increasing N assimilation to more than 50% (Hauck 1985; Lubkowski & Grzmil 2007; Shaviv & Mikkelsen 1993). A study by Wenyu et al. (2005) also revealed that controlled-release urea (CRU) can increase wheat grain yield, protein content and markedly reduce NO_3^- leaching. In addition, CRF (polyolefin-coated urea) was reported to reduce N_2O emission by ~81% in flood irrigated barley grown on a clay soil in north eastern Colorado, USA producing a total N recovery of 66% (Shoji et al. 2001). Other merits associated with SR/CR fertilisers include reduced anthropogenic eutrophication (Lubkowski & Grzmil 2007; Noellsch et al. 2009; Shoji et al. 2001), and significant savings in labour, time and energy as single fertiliser application at sowing is possible precluding the need for topdressing (Lubkowski & Grzmil 2007; Mathers et al. 2007; Oertli 1980; Shaviv & Mikkelsen 1993). Additionally, a 20-30% reduction in the recommended conventional fertiliser application rate has been suggested when applying SR/CR fertiliser without incurring any yield penalty (Trenkel 2010).

Despite continuing technological developments and commercial availability of SR/CR fertilisers, their widespread use in agriculture is still constrained (Mathers et al. 2007; Shaviv & Mikkelsen 1993), contributing only 10% of the total amount of fertiliser used (Chen et al. 2008). This limited adoption has been attributed to the high fertiliser cost, which is often 3-10 times higher than that of a conventional fertiliser (Chen et al. 2008; Heffer 2009; Noellsch et al. 2009; Shaviv 2001). Claims of infrequent yield increases despite the additional costs have also been highlighted as a major hindrance to the adoption of this technology (Akiyama et al. 2010). These occasional yield increases could presumably be due to the fact that small grain crops such as wheat remobilise N where large amounts of N absorbed in the early growth stages and stored in the leaves are later translocated to the developing seed resulting in efficient use of soluble N (Seward 1984). In Australia, the use of SR/CR fertilisers is also still limited (Chen et al. 2008; Mathers et al. 2007), despite reported potential to increase in crops' yield and profitability (Lubkowski & Grzmil 2007; Oertli 1980; Shaviv & Mikkelsen 1993). The slow adoption of this technology by farmers may be due to the limited research underscoring the importance of SR/CR fertilisers in broadacre cropping compared with non-agricultural crops such as turf grass (Shaviv & Mikkelsen 1993). The few studies conducted in cereals such as rice or barley, have been mainly focussed on nitrification inhibitors and their role in reducing N_2O emissions rather than improving NUE. In addition, these studies have been mainly conducted in China and Japan under various rice production systems (Akiyama et al. 2010; Mosier et al. 1996; Mosier et al. 1998). This presented a substantial knowledge gap, which this research sought to address by investigating how SR/CR N fertilisers compared with conventional N sources in improving NUE in wheat and barley. Under varying irrigation regimes, the study assessed the interaction between different N

sources and how soil moisture content impacts on their effectiveness particularly under waterlogged conditions.

2.5 Duplex soils

Duplex soils are widely distributed in Australia (Tennant et al. 1992), covering about 20% of the continent, see Figure 2.2 (Chittleborough 1992). They have three dominant soil orders i.e. Sodosols, Kurosols and Chromosols characterised with a clear or abrupt textural B horizon (Cotching et al. 2009; Hardie et al. 2012). Duplex soils occupy 80% of the agricultural regions in southern Australia (Hardie et al. 2012) and 60% of the south-west agricultural land in Western Australia (Bakker et al. 2005; Bakker et al. 2007; Cox & McFarlane 1995; Tennant et al. 1992; Turner 1992). In Tasmania, duplex soils occupy about 23% of the land mass, mainly in the lower rainfall areas of eastern Tasmania; see Figure 2.3 (Cotching et al. 2009). Although the term duplex is mainly used in Australia, such texture contrast soils are common in other parts of the world (Chittleborough 1992; Cox & McFarlane 1995; Hardie et al. 2012).

Duplex soils are associated with a range of management problems including: waterlogging, poor crop establishment, crusting, poor root penetration, desiccation, wind, water and tunnel erosion, salinity and poor nutrient status (Belford et al. 1992; Hardie et al. 2012; Zhang et al. 2004). These management problems mainly stem from their morphological features as they are characterised by a strong texture-contrast between the A and B horizons (Bakker et al. 2005; Belford et al. 1992; Chittleborough 1992; Cox & McFarlane 1995) and hydraulic properties (Bakker et al. 2005; Bakker et al. 2007; Fillery & McInnes 1992). Usually the B horizon contains at least 20% more clay than the A horizon, which can be sandy or sandy loam; the horizon boundaries range from clear to

sharp (Chittleborough 1992; Hardie et al. 2012). However, the chemical, mineralogical and physical properties are not diagnostic (Chittleborough 1992; Cox & McFarlane 1995).

Figure 2.2: Distribution of duplex soils in Australia. Adapted from Chittleborough (1992).

Figure 2.3: Distribution of Sodosols in Tasmania. Adapted from Cotching et al. (2009).

2.5.1 Waterlogging of duplex soils

Waterlogging, one of the most hazardous natural occurrences is a phenomenon often described as flooding, submergence, soil saturation, anoxia and hypoxia depending on the soil moisture content (Ahmed et al. 2012). In duplex soils, waterlogging is characterised by excess water within the crop root zone due to the development of a perched water table on the subsoil clay (Bakker et al. 2005; Condon et al. 2002; Cox & McFarlane 1995;

Zhang et al. 2006). Seasonally perched water tables are often caused by winter rainfall exceeding evapotranspiration, limited storage capacity and slow soil drainage rates due to low porosity of the subsoil, high gravel and clay content (Bakker et al. 2005; Bakker et al. 2007; Hardie et al. 2012; Pang et al. 2004; Zhang et al. 2006). Nonetheless, waterlogging can occur during autumn or spring (Vartapetian & Jackson 1997).

Waterlogging creates water-saturated soil conditions with inadequate gas diffusion, 10^4 times slower than in air (Colmer & Greenway 2011; Greenway et al. 2006; Ponnamperuma 1972), leading to the development of anaerobic conditions (Condon & Giunta 2003; Parent et al. 2008; Sairam et al. 2008; Trought & Drew 1980). This increases the concentration of CO_2, C_2H_4, Mn^{2+}, Fe^{2+} and anaerobic microbial metabolites and lowers the concentration of NO_3^- and SO_4^{2-} ions (Colmer et al. 2001; Malik et al. 2002; Setter et al. 2009; Trought & Drew 1980). The accumulation of CO_2 is due to a combination of a 34-fold higher solubility of CO_2 than O_2 in aqueous medium and the conversion of a substantial proportion of CO_2 to HCO_3^-, which is prominent at a higher soil pH (Greenway et al. 2006). Furthermore, under warm soil conditions respiration by microbes is stimulated which depletes O_2 within 24 hours (Drew 1997). This depletion of soil O_2 either completely (anoxia) or partially (hypoxia) (Crawford & Braendle 1996; Hossain & Uddin 2011), causes seminal root tip death, restricted nodal root length and limits plant nutrient uptake and translocation of photoassimilates (Colmer & Greenway 2011; Condon & Giunta 2003; Parent et al. 2008; Zhang et al. 2004), which reduces plant growth, development and survival (Hardie et al. 2012; Parent et al. 2008). The intensity of waterlogging is often low where topsoils are deeper than 0.6 m with the A_2 horizon having high hydraulic conductivity (>7.8 m day^{-1}) and a sloping site facilitating lateral flows (Cox & McFarlane 1995; Hardie et al. 2012).

2.5.2 Effect of waterlogging on cereal production

Waterlogging of duplex soils is a major abiotic constraint to cereal production in the HRZ of Australia (Acuña et al. 2011; Bakker et al. 2005; Bakker et al. 2007; Zhang et al. 2006), particularly in regions with duplex soils (Yaduvanshi et al. 2012). It is also increasingly becoming a matter of worldwide concern affecting 16% of soils in the United States of America (USA) and irrigated crop production areas of India, Pakistan and China (Colmer & Greenway 2011; Pang et al. 2004; Sairam et al. 2008). In addition, cereal production in East and Central Africa including the central highlands of Ethiopia is adversely affected by waterlogging both on irrigated heavy clay soils and Vertisols (Yaduvanshi et al. 2012). On a global scale, about 10-15 million ha of the world's wheat growing areas are affected annually by waterlogging, representing 15-20% of the 70 million ha cultivated annually (Hossain & Uddin 2011; Yaduvanshi et al. 2012). Waterlogging is estimated to reduce winter wheat grain yield by 20-50% (Collaku & Harrison 2002; Tan et al. 2008). For barley, yield reductions range between 20-25%, but losses may exceed 50% depending on the stage of plant development (Pang et al. 2004). In Australia, waterlogging causes an estimated annual production loss of AU$180 million (Pang et al. 2004); a greater proportion of this is incurred in Western Australia where it reduces spring wheat yield by ~30-55% (Zhang et al. 2006), causing AU$100 million in crop losses in a wet year (Zhang et al. 2004). In Tasmania, where the state enjoys relatively high rainfall coupled with a range of soil orders that are often saturated in winter, waterlogging is a major constraint to cereal production causing yield losses of 39-49% in wheat despite its relative tolerance compared with barley (Zhou 2010). These yield losses are usually as a result of restricted root growth, reduced tiller number, premature leaf senescence and production of sterile florets (Collaku & Harrison 2005; Hossain & Uddin 2011).

2.5.2.1 Effect of waterlogging on plant growth and development

Waterlogging adversely affects plant growth and various physiological processes (Pang et al. 2004). It reduces the uptake and availability of essential nutrients (Pang et al. 2007; Pang et al. 2004), leaving plants with marked N, P, K, Ca and Mg deficiency symptoms (Huang et al. 1994b; Trought & Drew 1980). The occurrence of these nutritional deficiency symptoms could be attributed to the substantial dilution of nutrient concentrations, which decreases the availability of these nutrients and increases the leaching of mobile nutrients (Huang et al. 1994b; Pang et al. 2007; Pang et al. 2004). Another factor could be the decrease in root biomass, which impairs the ability of plant roots to take up and transport mineral nutrients to the shoot (Huang et al. 1994b; Pang et al. 2007; Pang et al. 2004). The decrease in root biomass often occurs as a result of root death due to the anaerobic conditions within the root zone created by waterlogging. This causes lodging especially at ear emergence (Watson et al. 1976).

Anaerobic conditions also cause stomatal closure, which reduces intracellular CO_2 thus limiting carbon fixation in leaves (Elzenga & van Veen 2010; Sharma et al. 2011). Anaerobic conditions are due to waterlogging barring O_2 diffusion, which decreases cellular O_2 levels to concentrations that restrict aerobic respiration (Bailey-Serres & Voesenek 2008). The reduction in O_2 diffusion is due to the very small diffusion coefficient of O_2 in water compared with the gaseous phase (Vartapetian & Jackson 1997).

Various studies have investigated the effect of waterlogging on cereal growth under controlled conditions. In wheat, earlier studies by Trought and Drew (1980) on anaerobic solution cultures showed that waterlogging can damage young plants through premature senescence of older leaves and arrested growth of seminal roots. Hypoxic conditions inhibited shoot and root growth, reduced stomatal conductance and enhanced root

aerenchyma formation while resumption of aeration increased shoot and root growth and stomatal conductance (Huang et al. 1994a). The formation of aerenchyma occurs in the cortical tissue of both seminal and nodal roots (Huang et al. 1994b). Similarly, the work of Colmer et al. (2001) and Robertson et al. (2009), clearly indicated that waterlogging decreases relative shoot and root growth, net photosynthesis, tiller numbers and increases adventitious roots formed per stem by 1.5-fold. Subsequent recovery however, increases shoot growth as plants produce high-order tillers that develop late ears. In the same light, studies conducted by Malik et al. (2002) and Elzenga and van Veen (2010) further underscore the adverse effects of waterlogging, which inhibited seminal root growth and decreased leaf N content.

Studies on barley also reveal that indeed waterlogging can negatively impact on plant growth and development (Colmer 2015; Colmer & Greenway 2011; Malik et al. 2011; Pang et al. 2007; Pang et al. 2004). A study by Pang et al. (2004) showed that waterlogging has a negative effect on shoot and root growth, decreases leaf chlorophyll content, CO_2 assimilation rate and maximal quantum efficiency of photosystem II (Fv/Fm). It also inhibits net N uptake, which decreases shoot N concentration. The decrease in shoot N concentration could be due the translocation of N from older to younger expanding leaves, which causes chlorosis, accelerated leaf senescence and retarded growth thereby decreasing dry matter accumulation and final yield (Colmer et al. 2001; Drew & Sisworo 1977).

Under field conditions, limited research has been conducted to investigate the effects of waterlogging on wheat or barley growth and development. Crops under natural environmental conditions are often exposed to transient or permanent waterlogging (Ashraf 2012). Prolonged or permanent waterlogging can cause significant yield

reductions as the soil is completely depleted of O_2 (Parelle et al. 2010). In Australia, transient waterlogging is a common occurrence on sandy duplex soils, where rainfall rapidly penetrates the sandy topsoil and accumulates above the compacted clay subsoil with low hydraulic conductivity (Yaduvanshi et al. 2012). Like under controlled conditions, waterlogging under field conditions is reported to reduce root growth, production of tillers and fertile heads and delay ear emergence. This significantly impacts on the final biomass and grain yield (Belford et al. 1992; Watson et al. 1976). Transient waterlogging during winter and spring is said to cause significant wheat yield reductions in southern Australia (Condon & Giunta 2003). These yield reductions are associated with reduced production and survival of fertile tillers, reduced shoot N and chlorophyll content and accelerated senescence of older leaves. In the HRZ of south-western Australia, waterlogging decreased shoot dry matter at anthesis in wheat (Zhang et al. 2004). This causes remobilization of stored N from older leaves and roots to sustain the grain filling phase (Hirel et al. 2007).

Given this evidence, it can be seen that indeed waterlogging affects crop growth and development. However, limited research has been conducted to understand the impact of waterlogging on wheat and barley under field conditions. Although some research has been conducted in different HRZs of Australia, to our current knowledge no studies have been documented for Tasmania despite the acknowledgment of waterlogging as a serious constraint to wheat and barley production in the region. This is a key knowledge gap that this research sought to address.

Of note, the severity of the effects of waterlogging is dependent on its occurrence and intensity (Condon & Giunta 2003; Malik et al. 2002), and the crop developmental stage (Malik et al. 2002; Pang et al. 2004; Zhang et al. 2004). Usually, the early vegetative phase

is more affected than the late vegetative and reproductive phases (Zhang et al. 2006; Zhang et al. 2004). Similarly, the duration of waterlogging has been reported to increase its severity. For instance, Belford (1981) obtained wheat grain yield losses of 2% and 16% after 47 and 80 days of waterlogging, respectively. In addition, Yaduvanshi et al. (2012) investigated the influence of waterlogging on wheat yield, redox potentials and concentration of microelements on two Australian soils (a sandy-duplex and a neutral, sodic, sandy loam 'grey clay'). The authors observed a significant decline in wheat yield with the duration of waterlogging on sodic soils and redox potentials of ~210 mV were obtained at 10 days of waterlogging with a further decline of up to 100 mV after 40 days. Figure 2.4 presented below gives a description of the processes leading to reductions in plant growth and yields due to waterlogging.

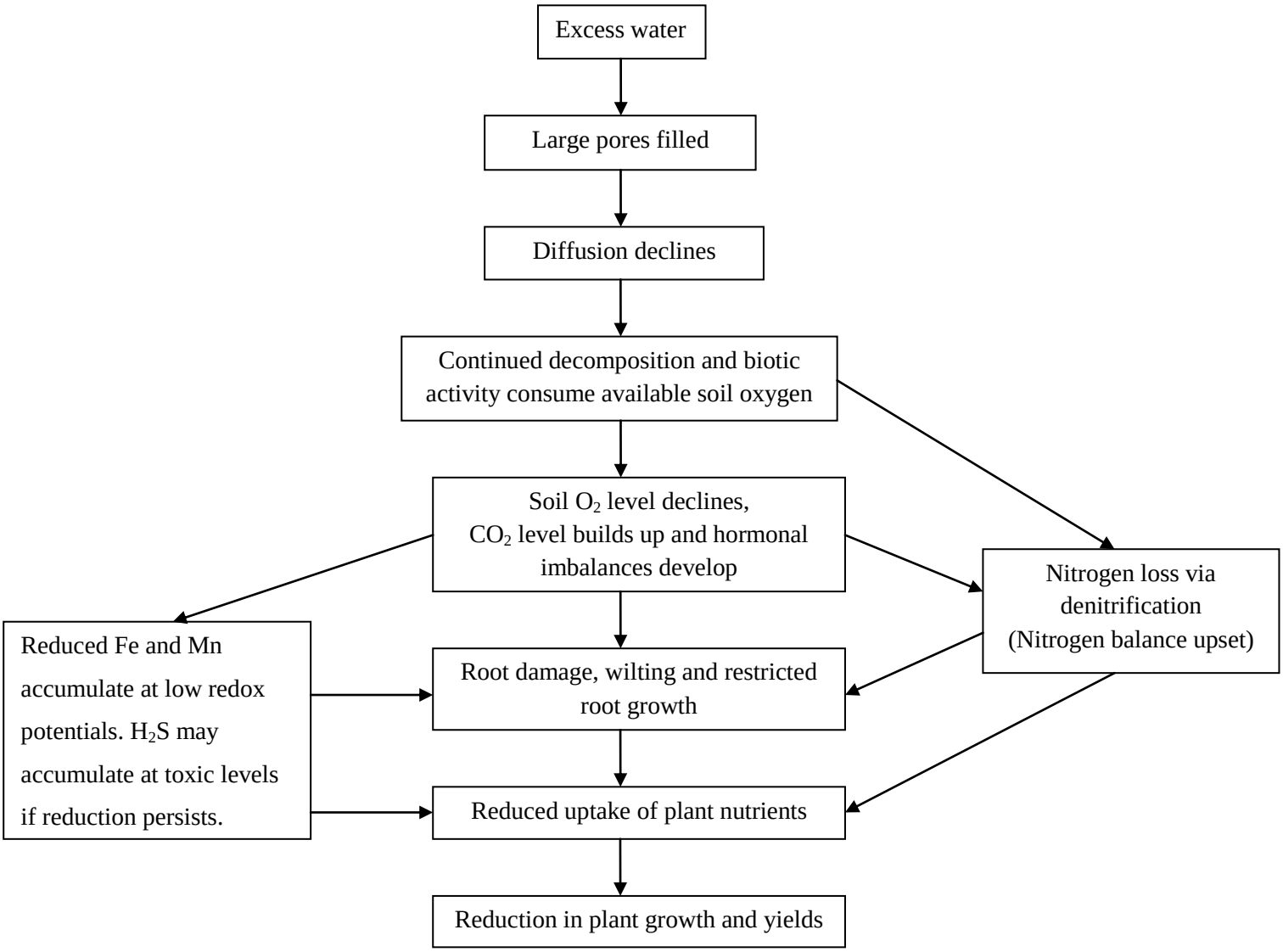

Figure 2.4: Summary of processes leading to reduction in plant growth and yields due to waterlogging. Adapted from Evans et al. (1999).

2.5.2.2 Effect of waterlogging on NUE

Waterlogging can significantly reduce NUE through denitrification and leaching yet NUE is an important measure of crop productivity in different environmental systems (Asseng et al. 2001). Moll et al. (1982) defined NUE as grain yield per unit of available N in the soil including the residual soil N and fertiliser. NUE can be divided into two components: Nitrogen-uptake efficiency (NupE); the ability of the plant to remove N from the soil as

NH_4^+ and NO_3^- and Nitrogen-utilization efficiency (NutE); the ability of the plant to use N to produce grain yield (Fischer et al. 2014; Lea & Azevedo 2006; Moll et al. 1982). Nitrogen harvest index (NHI) defined as N in grain per total plant N uptake (Hirel et al. 2007), reflects grain protein content and grain nutritional quality. The world wheat grain NUE is about 34%; however, this varies between 10-70% depending on the agro-ecological environment (Rane et al. 2011). The major determinants of NupE are uptake activity or plant demand and root architecture, which are facilitated by influx transporters located on the plasma membrane and alterations in primary and lateral root growth and development in response to local and systemic N signals, respectively (Kiba & Krapp 2016). NutE is largely determined by the efficiency of enzymes, nitrate reductase and nitrite reductase, which convert NO_3^- to NH_4^+ and ammonium transporters (AMTs) as well as the GS/glutamine-2-oxoglutarate aminotransferase (GOGAT) cycle, where ammonium is assimilated into amino acids (Lam et al. 1996; Xu et al. 2012).

Soil moisture plays a significant role in the uptake of soil nutrients. Interactions between N supply and water availability were discussed by Sadras et al. (2016). Cereal production in which N fertiliser application is coordinated with irrigation can increase NupE to ~65% (Wuest & Cassman 1992a). Appropriate irrigation scheduling is therefore vital to ensure maximum NUE (Grant et al. 1991; Hirel et al. 2007; Shangguan et al. 2000a). Optimum soil moisture content of 50-100% field capacity (FC) is recommended for efficient uptake of soil nutrients including N. However, for SR/CR N fertilisers whose nutrient release is largely dependent on soil temperature, moisture content of 50-100% FC has little effect on nutrient release rate (Huett & Gogel 2000; Kochba et al. 1990). On the other hand, limited and excessive water availability may affect the rate of N release. At 25% FC, nutrient release from SR/CR N fertilisers is usually slower and completely stops in dry soils (Kochba et al. 1990). Abiotic stresses such waterlogging, significantly impact on soil

nutrient availability and uptake efficiency. NUE can be reduced by waterlogging as significant amounts of N are lost through volatilization, denitrification and leaching (Ju et al. 2009; Noellsch et al. 2009). Trought and Drew (1980) and reviews by Sairam et al. (2008) and Parent et al. (2008), indicate that anoxia due to waterlogging inhibits nutrient uptake particularly NO_3^-, PO_4^{3-} and K^+ and depresses water uptake. This results in N deficiency, which reduces yields and resultant NUE (Swarup & Sharma 1993). Nitrogen deficiency also lowers the photosynthetic rate largely due to the reduction in LAI, LA duration and chlorophyll content, which decreases canopy stomatal conductance thus limiting intracellular CO_2 concentration (Cai et al. 2011; Fois et al. 2009; Latiri-Souki et al. 1998).

Arguably, waterlogging decreases N uptake, however, subsequent recovery of plants after waterlogging is said to improve N uptake (Pang et al. 2004), through the development of new roots and enhanced development of adventitious roots with aerenchyma tissues formed during waterlogging. With most waterlogging incidences being transient, the recovery period can counteract the adverse effects of waterlogging including yield setback depending on the crop growth stage, duration and severity or intensity of waterlogging. This increases grain yield (Belford et al. 1992) since waterlogging may just induce a transient N deficiency (Robertson et al. 2009), which is offset during recovery. Apart from the application of conventional N sources and may be relying solely on the recovery of plants to alleviate the adverse effects of waterlogging, SR/CR fertilisers can be used improve crop growth and NUE during waterlogging by synchronising N supply with crop demand, which reduces N loss through leaching and denitrification (Chen et al. 2008). Yet to our current knowledge limited research has been conducted to assess the potential of SR/CR fertilisers in improving plant growth and development and NUE after

waterlogging. Furthermore, though most studies indicate that waterlogging decreases N availability and N uptake, no studies have documented its definitive effect on NUE.

2.5.3 Strategies to alleviate the adverse effects of waterlogging

Waterlogging significantly affects soil nutrient availability; thereby impacting on plant growth and development. Nitrogen being one of the nutrients whose availability is affected by waterlogging; management strategies to mitigate the adverse effects of waterlogging through reduced N loss are viewed as a viable option (Belford 1981). These strategies include appropriate N fertiliser application timings and use of enhanced-efficiency N fertilisers such as SR/CR fertilisers (Dinnes et al. 2002). Nitrogen fertiliser application is said to play a significant role in improving plant growth and development under waterlogged conditions (Pang et al. 2007; Robertson et al. 2009; Swarup & Sharma 1993). Notwithstanding the role of N fertiliser application in ameliorating the adverse effects of waterlogging, no research has been conducted to exploit the potential of SR/CR fertilisers under waterlogged conditions. Most studies have focused on conventional N sources (Robertson et al. 2009; Swarup & Sharma 1993) and foliar applied N (Pang et al. 2007) with no deliberate intention to explore the potential of SR/CR fertilisers. Furthermore, most N is top-dressed after waterlogging, which can be difficult under field conditions as the soil moisture content is often too high for efficient mechanization. This presented an opportunity to investigate how SR/CR N fertiliser and conventional urea, both applied as a single application at sowing would affect plant growth, resultant yield and NUE after waterlogging.

Other agronomic practices to alleviate waterlogging effects on plant growth include the use of raised bed farming systems and surface and subsurface drains (‘spoon drains’, ‘W-drains’, interceptor and reverse interceptor drains) (Bakker et al. 2007; Zhang et al. 2006).

The application of plant growth regulators (PGRs) such as auxins and cytokinins is also one such strategy. The two hormones act in concert to promote stomatal conductance and photosynthetic capacity of waterlogged plants (Drew 1991).

Besides, plants are also known to forage for limiting resources by adjusting carbon allocation and overall plant architecture to consolidate resource capture (Bailey-Serres & Voesenek 2008). One such morphological modification is aerenchyma formation (Voesenek et al. 2006), which facilitates long-distance gas transport (Wenger 2010), by decreasing longitudinal resistance to O_2 diffusion. Aerenchyma tissues have large intracellular spaces to render low-resistance internal pathways for gaseous exchange between the aerobic shoot and the anaerobic root (Ahmed et al. 2012; Voesenek et al. 2006). There are three distinct types of aerenchyma and these include; (a) lysigenous aerenchyma formed through programmed cell death, (b) schizogenous aerenchyma developed through the separation of previously connected cells, and (c) expansigenous aerenchyma, which is characterized by intracellular gas spaces developed through cell division and cell enlargement without cell separation or death (Bailey-Serres & Voesenek 2008). It is worth noting that hypoxic conditions signalled by ethylene play a vital role in signalling cell death during aerenchyma formation (Drew 1997).

The development of numerous adventitious roots that grow horizontally at the shoot base in order to access oxygen is also a well-known coping mechanism (Colmer et al. 2001; Pang et al. 2007; Pang et al. 2004; Sairam et al. 2008). Because of their strategic location, adventitious roots enable gaseous diffusion between the roots and the shoots (Ahmed et al. 2012). Other physiological modifications include: increased availability of soluble sugars, greater activity of the glycolytic pathway and fermentation enzymes and involvement of antioxidant defence mechanisms to cope with hypoxia/anoxia oxidative stress (Ahmed et

al. 2012; Goggin & Colmer 2007; Hossain & Uddin 2011; Sairam et al. 2008). Furthermore, avoidance of self-poisoning by ethanol through alcohol fermentation and cytoplasmic acidosis and modification of gene expression are also some of the physiological mechanisms used to cope with such stress (Vartapetian & Jackson 1997). In rice where total submergence occurs, elongation of internodes results in an escape strategy characterised by enhanced growth rate to maintain the top of the shoot above the water level (Bailey-Serres & Voesenek 2008; Parelle et al. 2010; Vartapetian & Jackson 1997). Achieving the unusually fast shoot extension rate helps to maintain contact with the O_2 source (Vartapetian & Jackson 1997).

At a developmental level, plants can escape low O_2 stress through multifaceted alterations in cellular and organ structure to promote O_2 uptake. This amelioration response is called low oxygen escape syndrome (LOES) associated with enhanced elongation rate of petioles, stems or leaves (Bailey-Serres & Voesenek 2008; Voesenek et al. 2006). Concomitant with high elongation rates, leaves develop a thinner overall morphology, higher specific leaf area (SLA), thinner epidermal cell walls and cuticles and chloroplasts oriented towards the leaf surface (Colmer & Voesenek 2009). This reduces the resistance for diffusion of CO_2 and O_2 facilitating inward diffusion thereby improving underwater photosynthesis and aerobic metabolism (Bailey-Serres & Voesenek 2008; Voesenek et al. 2006). In some species, this increased shoot elongation is preceded by an adjustment in leaf angle to a more vertical position (Voesenek et al. 2006). Of note, adaptive morphological traits are slower to develop compared to physiological adjustments (Parelle et al. 2010). Physiological adaptive traits are often of utmost importance in plants under short-term waterlogging (Colmer & Voesenek 2009). Therefore, waterlogging tolerance is not a single plant attribute, but rather an amalgam of processes and features that enhance plant survival (Crawford & Braendle 1996; Elzenga & van Veen 2010; Perata & Alpi

1993). Figure 2.5 below shows a schematic presentation of morphological and metabolic adaptive traits for waterlogging in wheat.

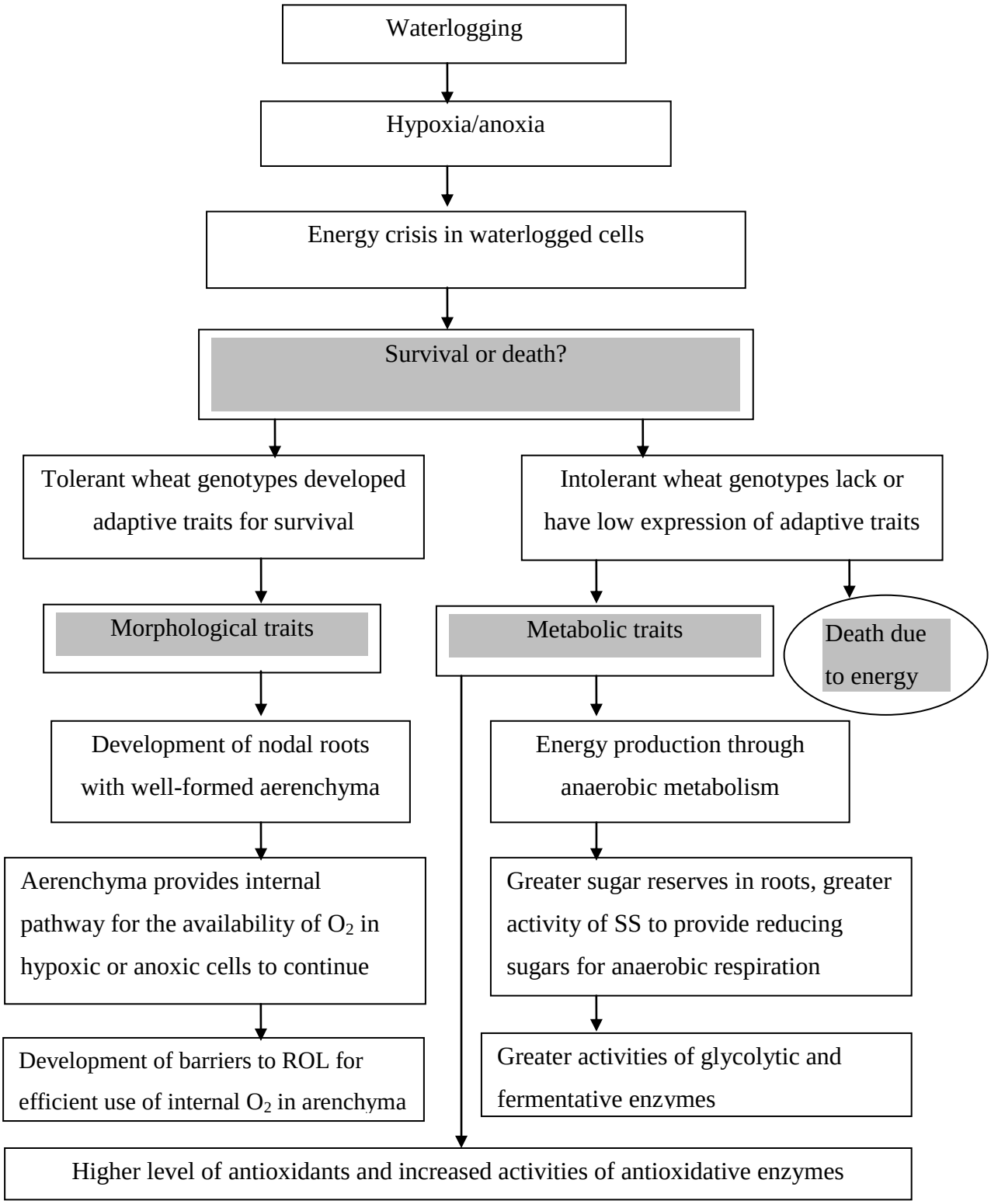

Figure 2.5: Schematic diagram showing morphological and metabolic adaptive traits for waterlogging in wheat. Adapted from Hossain and Uddin (2011), where SS: sucrose synthase, ROL: Radial Oxygen Loss.

2.6 Kinetics of nitrogen uptake in plants

In most soils, NH_4^+ and NO_3^- are the predominant sources of N available for plant nutrition (von Wirén et al. 2000). The charge of the ion strongly influences whether transport will be thermo-dynamically favourable or require the expenditure of additional metabolic energy. It also determines the methods needed to maintain electroneutrality at the root-soil interface (Haynes 1990; Henriksen et al. 1992). The metabolic cost of absorption and assimilation differs between NH_4^+ and NO_3^-, the reduction of NO_3^- to NH_4^+ requires an additional 15ATPs (Salsac et al. 1987). The assimilation of each ion affects the cytoplasmic pH differently. The incorporation of NH_4^+ generates H^+, whereas the reduction of NO_3^- results in a net alkalinisation of the cytosol (Henriksen et al. 1992). When both NH_4^+ and NO_3^- are present in a nutrient solution, NH_4^+ is often taken up preferentially to NO_3^- (Alexandre et al. 2011; Botella et al. 1994) for the synthesis of amino acids and proteins (Schrader et al. 1972). Though this can be species and treatment specific (Alexandre et al. 2011), higher rates of total N uptake often occur when both NH_4^+ and NO_3^- are present in a nutrient solution compared with each source alone, which leads to improved plant growth and development (Botella et al. 1994).

The uptake of NH_4^+ and NO_3^- follows Michaelis-Menten kinetics (K_m) (Botella et al. 1994) and differs between the mature and growing (meristematic and elongation) root regions (Colmer & Bloom 1998). It also varies significantly depending on plant species or cultivar, time of day, external N concentration and N status of the root tissue (Glass 2003). Genotypic differences in N uptake exist, with some genotypes showing preference for one

form of N over the other (Glass 1988). Plasticity of different plant species towards N source indicates that most species show higher preference for NH_4^+ especially under low nutrient availability (Tylova-Munzarova et al. 2005). This sensitivity to N forms depends on the rhizosphere N availability and pH (Tylova-Munzarova et al. 2005).

2.6.1 Kinetics of NH_4^+ uptake in plants

The uptake of NH_4^+ follows a biphasic pattern with a saturable carrier-mediated system operating at low $[NH_4^+]_0$ and a linear diffusive component at elevated $[NH_4^+]_0$ (von Wirén et al. 2000; Wang et al. 1993). NH_4^+ uptake at low $[NH_4^+]_0$ conforms to K_m while at higher values of $[NH_4^+]_0$ (>1 mM) a linear non-saturating uptake is evident (Glass 2003; von Wirén et al. 2000). Linearity of up to 40 mM was confirmed using $^{13}NH_4^+$ and $^{15}NH_4^+$ in rice (Glass 2003). Most plant species show K_m of NH_4^+ high-affinity transport system (HATS) of 10-180 µM (Taylor & Bloom 1998).

At low $[NH_4^+]_0$, NH_4^+ influx is mediated by a saturable HATS that exhibits high temperature coefficient (Q_{10}) values between 10 and 30^0C and significant sensitivity to metabolic inhibitors. At elevated $[NH_4^+]_0$ (between 1 and 40 mM), NH_4^+ influx increases in a linear fashion with increasing $[NH_4^+]_0$ (Wang et al. 1994). NH_4^+ uptake causes rapid depolarization of the plasma membrane demonstrating a biphasic response to external NH_4^+ concentration. The extent of depolarization is influenced by plant N status (Wang et al. 1996). Short-term influxes of $^{13}NH_4^+$ measured in intact roots of 3-week-old rice seedlings showed that below 1 mM external concentration, NH_4^+ influx is saturable possibly due to the HATS (Wang et al. 1993). Between 1 and 40 mM of NH_4^+, $^{13}NH_4^+$ influx showed a linear response due to a low-affinity transport system (LATS). $^{13}NH_4^+$ influxes by the HATS and to a lesser extent the LATS are energy dependent processes (Wang et al. 1993).

The uptake of NH_4^+ from the soil employs both the high- and low-affinity transport systems (HATS and LATS) (Glass 2003; von Wirén et al. 2000). The HATS and LATS are distinguished by their apparent K_m values. High-affinity is an important property of a transporter responsible for extraction of nutrients at low external concentrations. Low-affinity on the other hand correlates with high capacity, a crucial parameter for maintenance of large influxes at high external availability (von Wirén et al. 2000). NH_4^+ is positively charged and can enter the cell passively down the electrochemical gradient. However, NH_4^+ uptake against the electrochemical gradient at external NH_4^+ levels less than 500 µM requires an active HATS (Taylor & Bloom 1998). Kinetic properties vary depending on the intensity of NH_4^+ supply to which the plants are acclimatized, plants adapted to low NH_4^+ growth conditions have a much lower K_m than plants adapted to an abundant supply of NH_4^+ (Forde & Clarkson 1999). NH_4^+ uptake increases following a period of N deprivation, the re-supply of NH_4^+ results in small but significant increase in net NH_4^+ uptake, a phenomenon described as "induction" (Glass 2003; Glass et al. 2002). Often a modest increase in HATS for NH_4^+ is observed after N-starved root are re-supplied with NH_4^+ (Glass et al. 2002).

Efflux of NH_4^+ involves passive diffusion of NH_4^+ from cells as protonated NH_3 into the external solution (Forde & Clarkson 1999). The rate of NH_4^+ efflux is positively correlated with $[NH_4^+]$ (Forde & Clarkson 1999). The HATS plays a significant role in retrieving NH_4^+ lost from epidermal and cortical cells by efflux (Forde & Clarkson 1999). Although NH_4^+ has lower energy costs, many plant species show restricted growth under strict NH_4^+ nutrition and often develop NH_4^+ toxicity syndrome, which is associated with NH_4^+ accumulation in the tissues or diminished cation uptake (Tylova-Munzarova et al. 2005).

2.6.1.1 Molecular mechanisms mediating NH_4^+ uptake in plants

NH_4^+ is the predominant form of N in soils where nitrification is inhibited, particularly under waterlogged conditions (Colmer 2015; von Wirén et al. 1997). In most agricultural soils, NO_3^- concentrations can range between 0.5-10 mM while NH_4^+ concentrations are often 10 to 1000 times lower (rarely exceeding 50 µM) (von Wirén et al. 1997). However, this difference in soil concentrations does not reflect the uptake ratio of both forms since most plants have a preference for NH_4^+ when both forms of N are available (Kronzucker et al. 1999; Tylova-Munzarova et al. 2005; von Wirén et al. 1997). According to Marschner (2011), NO_3^- concentrations are generally 10 times that of NH_4^+ and this ratio is consistent for the pool of N available to plants across soil types.

The uptake of NH_4^+ in plants is mediated by the ammonium transporter/methylammonium permease (*AMT/MEP*) membrane proteins of the *AMT* superfamily (Kiba & Krapp 2016; Ludewig et al. 2007). The *AMT* gene family is subdivided according to their amino-acid sequences into subfamilies of *AMT1* genes, such as *LeAMT1*from tomato and *AtAMT2* from *Arabidopsis thaliana* (von Wirén et al. 2000). These transporters vary in their kinetic properties and regulatory mechanism (von Wirén et al. 2000). NH_4^+ uptake has two kinetically distinct components: a low-affinity non-saturable (LATS) and a high-affinity saturable component (HATS) (Kiba & Krapp 2016). The HATS is induced in N-starved roots and plays a central part in efficient N uptake under low availability (Kiba & Krapp 2016) while the LATS(s) are considered as 'work horses' that are active when conditions are conducive for NH_4^+ assimilation (von Wirén et al. 1997; von Wirén et al. 2000). High-affinity NH_4^+ transporters belong to the *AMT* family of transporters (Garnett et al. 2003; Gazzarrini et al. 1999; von Wirén et al. 2000). The first NH_4^+ transporter genes (*AMT*) were identified from yeast and *Arabidopsis* by functional complementation of a yeast

mutant defective in high-affinity NH_4^+ uptake (Gazzarrini et al. 1999). The role of plant genes in NH_4^+ nutrition is supported by the preferential expression of NH_4^+ transporters in root hairs (Gazzarrini et al. 1999; von Wirén et al. 2000) and up-regulation of their transcript levels under N limitation (Kiba & Krapp 2016). High-affinity NH_4^+ uptake in roots is regulated in relation to the physiological status of the plant at the transcriptional level and by substrate affinities of individual members of the *AMT1* gene family (Gazzarrini et al. 1999). The spatial heterogeneity of expression of the *AMT1* genes responsible for high-affinity NH_4^+ uptake has been expounded by Kiba and Krapp (2016). NH_4^+ influx studies using triple and quadruple mutants showed that about 90% of the high-affinity uptake capacity under N limitation is regulated by the *AtAMT1;1*, *AtAMT1;2* and *AtAMT1;3* transporters (Gazzarrini et al. 1999; Kiba & Krapp 2016). NH_4^+ transporters are regulated by N supply form, diurnal and temperature fluctuations (Gazzarrini et al. 1999; Xu et al. 2012), and have different NH_4^+ affinity levels and transport capacities indicative of their roles in planta (Kiba & Krapp 2016). A diagrammatic presentation of the plant genes encoding NH_4^+ transporters is given below (Figure 2.6).

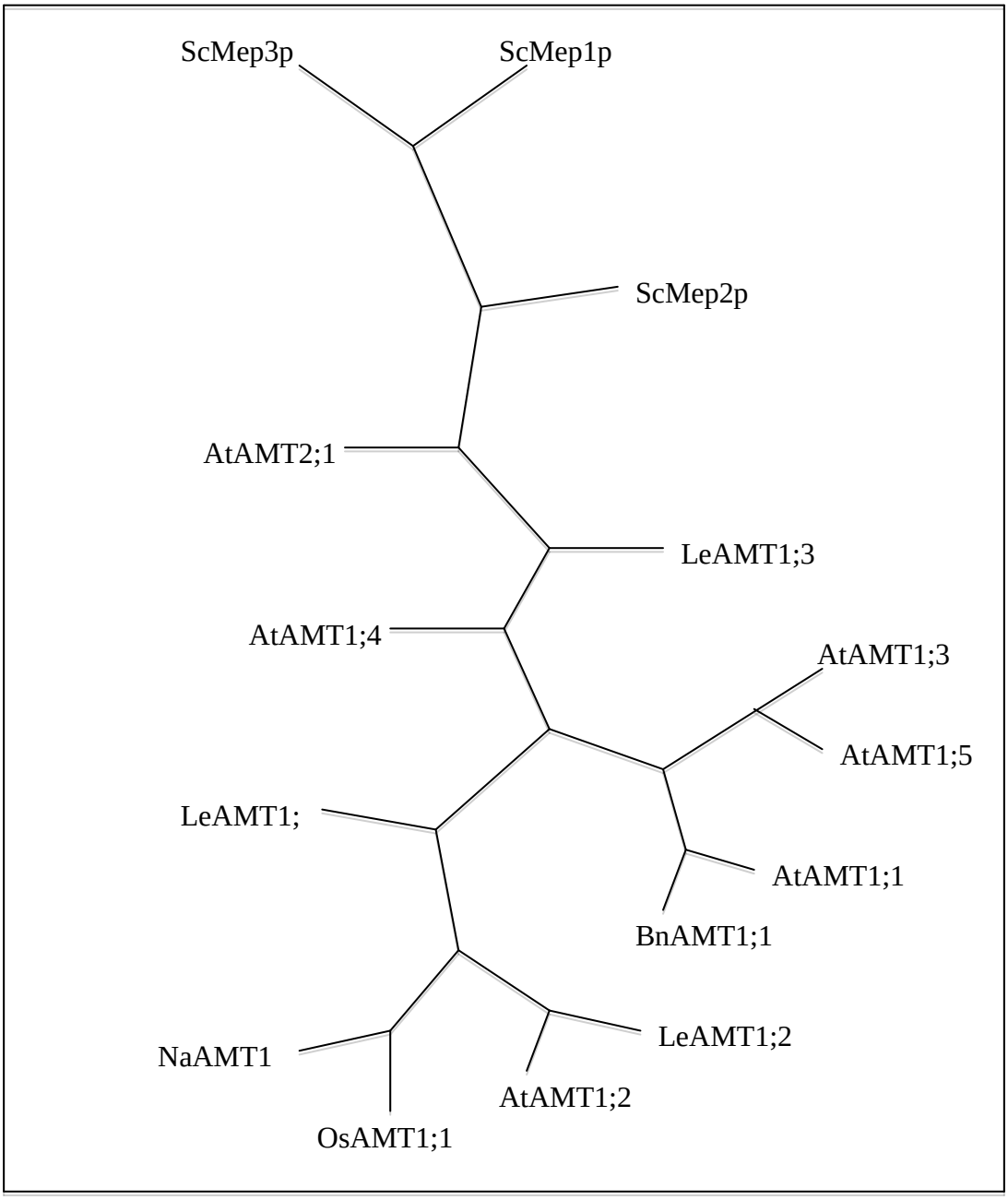

Figure 2.6: Phylogenetic tree of ammonium transporters of the *AMT/MEP* gene family in yeast (*Saccharomyces cerevisiae* [Sc]) and plants (*Arabidopsis thaliana* [At], *Lycopersicon esculentum* [Le], *Oryza sativa* [Os], *Brassica napus* [Bn] and *Nepenthes alata* [Na]). Adapted from Ludewig et al. (2007); von Wirén et al. (2000).

2.6.2 Kinetics of NO_3^- uptake in plants

Thermodynamic considerations indicate that NO_3^- uptake requires an active transport system. NO_3^- influx is proton-coupled and dependent on the H^+ pumping activity of the plasma membrane H^+-ATPase (Forde 2000). NO_3^- uptake also follows Michaelis-Menten kinetics and typically has a K_m of 17-50 μM; with a dual-phasic and possibly a multiphasic relationship (Hawkins & Lewis 1993; von Wirén et al. 1997). Kinetic studies show that roots have at least three-distinct NO_3^- uptake systems, two of which have a high affinity for NO_3^- and the third has a low-affinity (Okamoto et al. 2003). One of the high-affinity systems is strongly induced in the presence of an external NO_3^- supply and is known as the inducible high-affinity transport system (iHATS). The second high-affinity system is the constitutively expressed (cHATS) (Aslam et al. 1992; Crawford & Glass 1998; Daniel-Vedele et al. 1998; Forde 2000; von Wirén et al. 1997). The cHATS has a higher affinity for NO_3^- (Forde & Clarkson 1999), but iHATS has a much greater capacity for NO_3^- uptake (Crawford & Glass 1998). iHATS flux of fully induced plants is usually higher than that resulting from the cHATS (Crawford & Glass 1998). iHATS activity typically overshoots plant demand for NO_3^- uptake when first induced and is rapidly down-regulated after the initial exposure to NO_3^- (Crawford & Glass 1998). The iHATS is negatively feedback-regulated by the products of N assimilation (Forde 2000). Several forms of N including NO_3^-, NH_4^+ and amino acids may participate in the down-regulation of iHATS, which occurs at the mRNA level (Crawford & Glass 1998; King et al. 1993). Such regulation is important for coordinating root N uptake with shoot N demand.

The low-affinity transport system (LATS), constitutively expressed is important at external concentrations >1 mM and despite displaying linear kinetics it has an active H^+-dependent transport system (Aslam et al. 1992; Forde 2000; Glass 2003; Kronzucker et al. 1995;

Siddiqi et al. 1990). LATS like the iHATS is a proton- mediated system operating with a H^+:NO_3^- stoichiometry of 2:1 (Forde & Clarkson 1999). The LATS is also negatively feedback-regulated (Forde 2000).

Net NO_3^- uptake is determined by changes in both influx and efflux at the plasma membrane. The efflux of NO_3^- increases with increasing ambient [NO_3^-]. At high ambient [NO_3^-], efflux kinetics dominates patterns of net NO_3^- uptake (Glass 1988). NO_3^- efflux may also be a substrate inducible process that requires synthesis of both RNA and protein(s). Unlike the uptake system, the efflux system once induced is relatively stable. NO_3^- efflux from root cells plays a significant role in determining the net rate of NO_3^- uptake. Sometimes, 80% of the NO_3^- influx can be simultaneously dissipated by efflux (Forde & Clarkson 1999). The rate of NO_3^- efflux depends on both the external [NO_3^-] and relative growth rate of the plant, and rises sharply when influx exceeds the demand for N by the plant (Forde & Clarkson 1999).

2.6.2.1 Molecular mechanisms mediating NO_3^- uptake in plants

A large proportion of the NO_3^- acquired by plants from the soil is actively transported via members of the *NRT* families of NO_3^- transporters (Crawford & Glass 1998; Plett et al. 2010). The first NO_3^- transporters to be identified were the high-affinity crnA in *Aspergillus nidulans* and the low-affinity *CHL1* in *Arabidopsis thaliana*, both cloned using mutants defective in NO_3^- transport (Daniel-Vedele et al. 1998; Forde & Clarkson 1999). These findings were supported by Vidmar et al. (2000) and Yan et al. (2011). Molecular evidence has documented the existence of two families of genes encoding NO_3^- transporters among eukaryotes, namely the *NRT1* (*NPF*) and *NRT2* families (Nacry et al. 2013; Xu et al. 2012), which correspond to the physiological categories of low- and high-affinity NO_3^- transporters (Crawford & Glass 1998; Forde 2000; Okamoto et al. 2003;

Orsel et al. 2002b). In *Arabidopsis*, the *NRT1* family has eight functionally characterized members of the 53 members predicted from the genome sequence and predominantly comprises of low-affinity transporters (Kiba & Krapp 2016; Plett et al. 2010). The *NRT2* family contains seven members that appear to be high-affinity transporters; and there are two *NRT3* (*NAR2*) family members that are known to participate in high-affinity transport (Kiba & Krapp 2016; Plett et al. 2010).

The *NRT1* family comprises predominantly of low-affinity NO_3^- transporters, with the exception of *AtNRT1.1* that mediates dual-affinity NO_3^- transport based on phosphorylation status of the amino acid residue T101 (Liu & Tsay 2003). *AtNRT1.1* also functions as a NO_3^- and plays crucial role in plant proton tolerance by alkalifying the rhizosphere (Fang et al. 2016). The expression of *AtNRT1.2* is constitutive and located predominantly in the root epidermis indicating that the encoded transporter may be involved in NO_3^- uptake from the soil (Huang et al. 1999). *AtNRT1.3* expression in roots is repressed by exposure to NO_3^- and induced by NO_3^- deprivation (Okamoto et al. 2003; Orsel et al. 2002a; Orsel et al. 2002b). *AtNRT1.4* is expressed primarily in the leaf petiole and appears to be involved in NO_3^- storage (Chiu et al. 2004). *AtNRT1.5* mediates NO_3^- efflux and has a role in loading NO_3^- into the xylem for transport to the shoot (Lin et al. 2008). In *Arabidopsis*, *AtNRT1.6* is involved in transporting NO_3^- from the maternal tissue to developing embryos (Almagro et al. 2008). *AtNRT1.7* plays a role in the remobilization of NO_3^- from older to younger leaves through facilitating phloem loading (Fan et al. 2009). *AtNRT1.8* is responsible for retrieving NO_3^- from the xylem parenchyma in the roots and shoots, thus working synergistically with *AtNRT1.5* to control long-distance NO_3^- transport (Li et al. 2010; Plett et al. 2010). *AtNRT1.9* in root companion cells facilitates the loading of NO_3^- in the root phloem and enhances downward NO_3^- transport in roots (Xu et al. 2012).

The *NRT2* family are high-affinity NO_3^- transporters comprising of NO_3^- inducible and constitutively expressed members (O'Brien et al. 2016; Tsay et al. 2007). Of the seven *NRT2* genes in *Arabidopsis thaliana*, *AtNRT2.1*, *AtNRT2.2*, *AtNRT2.4* and *AtNRT2.5* are expressed in the roots of N deprived plants (Kiba & Krapp 2016; Li, W et al. 2007; Okamoto et al. 2003; Orsel et al. 2002b). Analysis of a quadruple mutant revealed that these four *NRT2* transporters account for ~95% of high-affinity NO_3^- influx activity under N limitation, *AtNRT2.1* being the major contributor (Lezhneva et al. 2014). During N deprivation, the expression of *AtNRT2.1* is transiently derepressed in the cortex cells of older parts of primary and lateral roots (Wirth et al. 2007). In contrast, the transcript levels of *AtNRT2.4* and *AtNRT2.5* increase during N deprivation over time in the epidermal cells of young primary and lateral roots (Kiba & Krapp 2016; Okamoto et al. 2003; Orsel et al. 2002b). These spatial expression patterns indicate that *AtNRT2.4* and *AtNRT2.5* are responsible for NO_3^- uptake from the soil, and *AtNRT2.1* is crucial for apoplastic NO_3^- absorption (Kiba & Krapp 2016; Lezhneva et al. 2014). Although *AtNRT2.4* and *AtNRT2.5* are expressed in the same cell types, the former is predominant in young seedlings and the latter in adult plants (Lezhneva et al. 2014). In addition, *AtNRT2.4* was suggested to have much higher affinity for NO_3^- than *AtNRT2.1* (Kiba & Krapp 2016). The expression of *AtNRT2.6* remains relatively unchanged in roots and shoots following exposure of plants to NO_3^- (Okamoto et al. 2003; Orsel et al. 2002b), while *AtNRT2.7* has a role in the storage of NO_3^- in seeds (Chopin et al. 2007). The *NRT2* family genes have also been investigated in other plant species, and many N-limitation-inducible genes have been identified (Kiba & Krapp 2016). Biochemically, they seem to act as high-affinity NO_3^- transporters; however, the functional characterization of these genes in planta remains to be carried out (Kiba & Krapp 2016).

Some high-affinity NO_3^- transporters belonging to the *NRT2* family need a partner protein *NAR2* for their function (Kiba & Krapp 2016; Yan et al. 2011). *NRT2.1* and *NAR2.1* polypeptides interact directly at the plasma membrane to constitute an oligomer that could be the functional unit for high-affinity NO_3^- influx in *Arabidopsis* roots (Yan et al. 2011). In barley, there are estimated to be at least seven members of the *NRT2* gene family; prolonged exposure of Northern blots showed that *NRT2* mRNAs are present in roots of barley seedlings that have never been exposed to external NO_3^- (Forde & Clarkson 1999). Therefore, one or more members of the *NRT2* family could be responsible for NO_3^- transport activity in uninduced roots (Forde & Clarkson 1999). A summary of the genes in the *NRT1* and *NRT2* families is shown in Tables 2.1 and 2.2 below, modified from Crawford and Glass (1998); Nacry et al. (2013).

Table 2. 1: *NRT1* family of nitrate transporters

NRT1(*NPF*) gene	Organism	Substrate	Regulation
AtNRT1 (CHL1)	*Arabidopsis*	NO_3^-	NO_3^-/acid inducible
AtNRT3 (NTL1)	*Arabidopsis*	NO_3^-	Constitutive
LeNRT1;1	Tomato	Unknown	Constitutive
LeNRT1;2	Tomato	Unknown	NO_3^- inducible
BnNRT1;2	*Brassica napus*	NO_3^- and basic AAs	NO_3^- inducible
AtPTR2B	*Arabidopsis*	Peptides	N/A
PepT1	Rabbit	Peptides	N/A
ScPTR2	*Saccharomyces*	Peptides	N/A

Table 2. 2: *NRT2* family of nitrate transporters

NRT2 gene	Organism	Substrate	Regulation
HvNRT2A	Barley	NO_3^-	NO_3^- inducible
HvNRT2B	Barley	NO_3^-	Unknown
AtNRT2;1	*Arabidopsis*	Unknown	NO_3^- inducible
AtNRT2;2	*Arabidopsis*	Unknown	NO_3^- inducible
GmNRT2	*Soybean*	Unknown	NO_3^- inducible
NpNRT2;1	*Nicotiana*	Unknown	NO_3^- inducible
Nrt2;1	*Chlamydomonas*	NO_3^- and NO_2^-	NO_3^- inducible
Nrt2;2	*Chlamydomonas*	NO_3^-	NO_3^- inducible
CRNA	*Aspergillus*	NO_3^-	NO_3^- inducible
YNT1	*Hansenula*	NO_3^-	NO_3^- inducible

The *NRT3* genes in *Arabidopsis* play a role in NO_3^- transport through regulating the activity of *NRT2* genes, but themselves are not transporters (Okamoto et al. 2006; Orsel et al. 2006). Two *NRT3* genes are closely related but *NRT3.1* plays a more significant role in high-affinity NO_3^- uptake (Okamoto et al. 2006). The *NRT3* also known as the *NAR2* family includes *OsNAR2.1*, which is expressed in roots, induced by NO_3^- and suppressed by NH_4^+ and some amino acids. *OsNAR2.1* interacting with *OsNRT2.1*/*OsNRT2.2* and *OsNRT2.3a* plays a key role in enabling plants to cope with variable NO_3^- supply (Yan et al. 2011).

2.7 Nitrogen uptake under hypoxia

Although both NH_4^+ and NO_3^- are important sources of N for plant growth, NH_4^+ preference is common in plants occupying habitats with restricted nitrification (Garnett et al. 2001; Kronzucker et al. 2000; Kronzucker et al. 1998; Kronzucker et al. 1995; Tylova-Munzarova et al. 2005; Wang et al. 1993; Yan et al. 2011). Anaerobic conditions cause substantial reduction in net NO_3^- uptake by roots of higher plants (Glass 1988) due to

denitrification and leaching (Buwalda & Greenway 1989). However, they can also reduce NH_4^+ uptake by ~ 50% (Bradley & Morris 1990). Nitrogen uptake was reduced by 16% in wheat grown in a nutrient solution containing a mixture of NH_4^+ and NO_3^- under hypoxia (Buwalda et al. 1988; Buwalda & Greenway 1989). Nitrogen uptake under hypoxia is sometimes limited by H_2S and elemental toxicity or salinity (Morris 1980).

2.7.1 Ammonium uptake under hypoxia

There is ample evidence that NH_4^+ can be absorbed and utilized directly by many plant species of higher plants (Arnon 1937; von Wirén et al. 2000). However, NH_4^+ uptake at concentrations less than 500 µM occurs against an electrochemical gradient (Taylor & Bloom 1998), which is reduced by hypoxia (Buwalda & Greenway 1989). A time-dependency study of NH_4^+ influx into rice roots after the onset of hypoxia showed an initial increase in NH_4^+ uptake followed by a 50% decline (Kronzucker et al. 1998). The importance of NH_4^+ as a N source under hypoxic conditions is vital due to: adsorption of NH_4^+ to soil colloids, while NO_3^- is readily leached; slow mineralization of organic N and; cessation of nitrification and denitrification of NO_3^- (Buwalda & Greenway 1989). The HATS induced in N starved roots (von Wirén et al. 2000) plays an important role in N acquisition under flooded anaerobic conditions when NH_4^+ is dominant and significant nitrification occurs on the root surface (Feng et al. 2011).

2.7.2 Nitrate uptake under hypoxia

NO_3^- is a major source of N for the vast majority of plants. It is reduced to nitrite (NO_2^-) by nitrate reductase (NR); the NO_2 is further reduced to NH_4^+ by nitrite reductase (NiR) under hypoxia (Bailey-Serres & Voesenek 2008; Daniel-Vedele et al. 1998). This makes NH_4^+ the major form of mineral N in waterlogged soils though in the rhizosphere of roots with radial oxygen loss (ROL), NH_4^+ can be converted back to NO_3^-, which are both absorbed

by the roots (Colmer 2015). NO_3^- is used as an alternative electron acceptor by some soil microorganisms during periods of partial anaerobiosis allowing the electron transport system (ETS) and oxidative phosphorylation to continue (Colmer 2015; Drew 1991). In O_2 deficient roots, NO_3^- ions may enter passively and translocate to the shoot in sufficient amounts to be of temporary benefit (Trought & Drew 1981). This temporary benefit stimulates NADH-dependent NR activity thereby diverting NADH from the reduction of acetaldehyde to ethanol, whose accumulation could damage sensitive tissues (Drew 1991).

Understanding the uptake of N and how this varies along the different physiological root zones under hypoxia requires a detailed study of nutrient fluxes. While a few studies have looked at the flux of NH_4^+ and NO_3^- ions in wheat and barley; most paid no attention to NH_4^+ and NO_3^- uptake under hypoxia and how this varies along the root axis. This research investigated to what extent NH_4^+ and NO_3^- uptake is affected by hypoxia along the root axis of selected wheat and barley varieties using the non-invasive microelectrode ion flux estimation (MIFE) technique. The MIFE technique offers a unique temporal and spatial pattern of the ions uptake, permitting detailed examination of inorganic N acquisition and its component ionic interactions (Henriksen et al. 1990).

2.7.3 The inhibitory effect of NH_4^+ on NO_3^- uptake in plants

The rate of NO_3^- uptake can be suppressed in the presence of NH_4^+ ions (Criddle et al. 1988; Tylova-Munzarova et al. 2005; Youngdahl et al. 1982). This suppression can be both short term and long term (Crawford & Glass 1998; Glass 2003; Orsel et al. 2002a). The short term effect is apparent within minutes of exposure to NH_4^+ while the long term can range from hours to days. The short term effect is attributed to the direct inhibition of NO_3^- uptake and stimulation of its efflux (Glass 2003; Kronzucker et al. 1999). This immediate inhibitory effect of NH_4^+ is exacerbated in the long term by the rapid

conversion of absorbed NH_4^+ to glutamine, a potent feedback inhibitor of transcription of the putative iHATS transporter gene *NRT2.1* (Glass 2003).

NH_4^+ markedly inhibits the uptake of NO_3^- in barley and wheat roots; it generally exceeds NO_3^- uptake from equimolar solutions (Minotti et al. 1969; Newman 2001; Taylor & Bloom 1998). This creates a high concentration of NH_4^+ and H^+ adjacent to the cellular boundary membranes, which modifies the permeability of the membranes to NO_3^- (Minotti et al. 1969). A study using $^{13}NO_3^-$ in barley showed that influx diminishes and efflux increases within minutes of exposure to NH_4^+ due to depolarisation of the plasma membrane. The depolarisation reduces the proton motive force (PMF) for active NO_3^- uptake by the $2H^+/1NO_3^-$ symport mechanism (Crawford & Glass 1998). In rice, compartmental analysis by efflux and ^{13}N radiotracer showed that NO_3^- influx and metabolism are strongly repressed by NH_4^+ (Kronzucker et al. 1999).

The extent of NH_4^+ suppression on NO_3^- uptake depends on the ionic concentration in the solution (Criddle et al. 1988). For instance, NH_4^+ of 100-500 µM inhibited net NO_3^- uptake in barley seedlings at a NO_3^- concentration of 10 µM but not at 100 µM (Criddle et al. 1988; Deane-Drummond & Glass 1983). Exposure to 1 mM NH_4^+ strongly reduces the influx of NO_3^- in uninduced plants particularly when the external concentration of NO_3^- is low (Kronzucker et al. 1999). At higher $[NO_3^-]$ and in induced plants the inhibitory effect of NH_4^+ diminishes an indication that NH_4^+ inhibition of NO_3^- uptake is mediated via effects on the iHATS rather than the cHATS or the LATS (Kronzucker et al. 1999).

The severity of NH_4^+ restriction of NO_3^- uptake can be moderated in the presence of K^+ (Pan et al. 1985; Rufty et al. 1982), which facilitates the flow of H_2O and NH_4^+ assimilates (potential negative assimilates) through the root symplasm to the xylem (Pan et al. 1985). Plants efficient in translocating negative effectors away from sites of NO_3^- uptake or

reduction exhibit minimal inhibition of NO_3^- uptake by NH_4^+ (Pan et al. 1985). The influx of NO_3^- can also be improved by induction through pre-treatment with NO_3^- (Newman 2001), though NO_3^- uptake is strongly reduced by prior accumulation of NO_3^- in the roots (Breteler & Siegerist 1984; Deane-Drummond & Glass 1983). Of note, the inhibition of NO_3^- accumulation in the root tissue and translocation via xylem vessels varies with genotype and root age (Criddle et al. 1988; Pan et al. 1985).

2.8 Conclusions

Plants take up both NO_3^- and NH_4^+ as the main N sources (Yan et al. 2011). However, NH_4^+ is a predominant form of N under anaerobic conditions (Yan et al. 2011). The uptake of NH_4^+ and NO_3^- is mediated by dual phasic and single systems, respectively (Goyal & Huffaker 1986) depending on the ionic concentration (Criddle et al. 1988). The kinetics and energy consumption involved in NO_3^- uptake differs from that of NH_4^+ (Hawkins & Lewis 1993). Although NH_4^+ has an inhibitory effect on NO_3^-, uptake of NO_3^- can be enhanced by induction.

The heterogeneous presence of N in the soil demands plants to evolve mechanisms to regulate NO_3^- and NH_4^+ influx (Glass et al. 2002). The possession of HATS and LATS enables them to cope with low or high NH_4^+ and NO_3^- concentrations in the soil (Crawford & Glass 1998; Feng et al. 2011; Forde 2000; Taylor & Bloom 1998). NO_3^- transporters, *NRT1* and *NRT2* contribute to the LATS and HATS for both uptake and distribution of NO_3^- (Feng et al. 2011; Plett et al. 2010). High-affinity NH_4^+ transporters belonging to the *AMT* family are induced in N-starved roots for NH_4^+ uptake (Garnett et al. 2003; Gazzarrini et al. 1999; von Wirén et al. 2000). To improve NUE, a more complete understanding of NH_4^+ (von Wirén et al. 2000), and NO_3^- (Plett et al. 2010), transport from the soil to the plant and within the plant is vital.

Hypoxia decreases the status of N in plants (Buwalda & Greenway 1989). O_2 deprivation has an immediate and substantial effect on root ion flux patterns and this effect differs in hypoxia intolerant and hypoxia tolerant wheat and barley varieties (Colmer & Greenway 2011). Most electrophysiological studies have focused on physiological and molecular mechanisms associated with the uptake of NH_4^+ under hypoxia particularly in rice (*Oryza sativa*) and model plant *Arabidopsis thaliana* (Crawford & Glass 1998; von Wirén et al. 2000). Little attention has been given to the uptake of NH_4^+, NO_3^- and H^+ along the root axis in other cereals such as wheat or barley under hypoxia. Understanding N uptake under hypoxia is vital to designing and developing management and mitigation strategies to improve N uptake efficiency and resultant NUE under waterlogged conditions. Such strategies include breeding waterlogging tolerant wheat and barley cultivars with desirable root morphological characteristics and architecture and growth habits to maximise N uptake and crop productivity. The physiological component of this research mapped the uptake of NH_4^+, NO_3^- and H^+ ions across the primary root axis of selected wheat and barley varieties.

Likewise, little has been done to examine the response of cereals to different N sources under waterlogged conditions. Though some studies have been done to mitigate waterlogging effects through nutrient application, little is known about the potential of SR/CR fertilisers to improve NUE particularly under waterlogged field conditions.

In light of the literature reviewed and conclusions drawn, there are compelling reasons for increasing NUE in agricultural systems to reduce environmental impacts while increasing yields and maintaining or decreasing N inputs. Efficient N fertiliser management is critical for the economic production of cereals and the long-term protection of the environment (López-Bellido et al. 2006). New technologies employing SR/CR fertilisers can be used as

effective mitigation alternatives to control environmental impacts of fertilization (Shoji et al. 2001). The agronomy component of this research investigated how N fertiliser application can ameliorate the adverse effects of waterlogging with focus on different N sources and different timings of N application. The conventional N fertiliser, urea and a SR/CR fertiliser were applied as single or split N applications.

Chapter Three: Can nitrogen fertiliser application alleviate the adverse effects of waterlogging in cereals?

3.1 Abstract

Selected wheat and barley varieties were assessed for their tolerance to waterlogging and susceptible varieties were used to investigate how controlled-release fertiliser (CRF) compares with conventional split-applied urea in improving plant growth under waterlogged conditions. The varieties were subjected to prolonged waterlogging instigated at the two leaf stage for 70 days. Different attributes for shoot vigour namely: tiller number, number of green leaves, leaf chlorophyll content and number of dead leaves were recorded fortnightly. The above-ground dry matter (AGDM) was determined after waterlogging. In the subsequent experiment, leaf chlorophyll content and chlorophyll fluorescence were recorded weekly and tiller number, green leaf area, AGDM and root DM were determined after waterlogging and recovery. The results showed significant variations between wheat and barley varieties ($P < 0.05$). Tiller number and the number of green leaves were markedly reduced by waterlogging particularly in wheat. Mackellar was the most affected with an 86% reduction in tiller number compared with other wheat varieties. Barley varieties, TX9425 and Naso Nijo had relatively similar number of tillers and green leaves for both the control and waterlogged. Leaf chlorophyll content and AGDM of both wheat and barley varieties were significantly affected by waterlogging ($P < 0.05$). Mackellar and Naso Nijo had the lowest amount of leaf chlorophyll content and AGDM for both wheat and barley, respectively. In the second experiment, N fertiliser application improved leaf chlorophyll content, tiller number, green leaf area, AGDM and root DM during waterlogging and recovery. Differences between N treatments were

evident for most of the parameters measured. After waterlogging, the CRF had the highest amount of AGDM for both Mackellar and Naso Nijo. It sustained N supply throughout the duration of the experiment. Conventional urea on the other hand, improved Mackellar and Naso Nijo's growth at the start of waterlogging and significantly during recovery as the remaining urea was top-dressed.

Key words: duplex soils; enhanced-efficiency fertilisers; *Hordeum vulgare* L.; *Triticum aestivum* L.; waterlogging; waterlogging tolerance

3.2 Introduction

Waterlogging of duplex soils is a major abiotic constraint to cereal production in the high rainfall zones of Australia (Acuña et al. 2011). It is estimated to cause yield losses of *ca*. 30-50% (Zhou 2010). In Tasmania, waterlogging occurs especially during winter due to rainfall exceeding evapotranspiration (Bakker et al. 2007; Hardie et al. 2012). This is exacerbated by the duplex nature of Tasmanian soils occupying 23% of the total land mass (Cotching et al. 2009). Waterlogging can be transient, intermittent, prolonged or permanent (Ashraf 2012). Transient waterlogging is widespread on duplex soils (Condon & Giunta 2003; Setter et al. 1999; Yaduvanshi et al. 2012). It is highly variable and depends on several factors including: the frequency and intensity of rainfall events, soil type, depth of the A-horizon and slope of the land (Condon & Giunta 2003).

Waterlogging adversely affects plant growth and development (Pang et al. 2004). It reduces the availability and uptake of essential nutrients (Pang et al. 2007; Pang et al. 2004), leaving plants with marked nutritional deficiency symptoms (Huang et al. 1994b; Trought & Drew 1980) that lead to yield losses (Collaku & Harrison 2005). Plants can modify their root architecture to maximize resource capture (Bailey-Serres & Voesenek 2008). Such modifications include: aerenchyma formation to facilitate long-distance gas

transport between the aerobic shoot and the anaerobic root (Wenger 2010), development of numerous adventitious roots at the shoot base (Colmer et al. 2001; Pang et al. 2007; Pang et al. 2004; Sairam et al. 2008) and elongation of internodes as an escape strategy (Bailey-Serres & Voesenek 2008; Parelle et al. 2010; Vartapetian & Jackson 1997). These mechanisms vary depending on the crop, growth habit of the cultivar and duration of waterlogging (Setter et al. 1999). Wheat (Condon & Giunta 2003) and barley (Pang et al. 2007; Pang et al. 2004) are sensitive to waterlogging; however, genetic diversity in waterlogging tolerance exists within both species (Setter et al. 1999; Thomson et al. 1992).

Agronomic management strategies to mitigate the adverse effects of waterlogging particularly reduced nitrogen (N) loss are also viable options. Nitrogen loss can be reduced through appropriate timing of N fertiliser application and use of enhanced-efficiency N fertilisers such as controlled-release fertilisers (CRFs) (Dinnes et al. 2002). Controlled-release fertilisers release N over an extended period of time during crop growth (Lubkowski & Grzmil 2007; Shaviv & Mikkelsen 1993; Trenkel 2010) and maximize nitrogen-use efficiency (NUE) by synchronizing N release with crop demand (Shaviv & Mikkelsen 1993; Trenkel 2010).

The application of N fertilisers plays a significant role in improving plant growth and development under waterlogged conditions (Pang et al. 2007; Swarup & Sharma 1993). For example, foliar applied N improved leaf chlorophyll content, net CO_2 assimilation, shoot and root growth and increased the production of adventitious roots in barley (Pang et al. 2007). Increased rates of top-dressed urea were reported to reduce flooding effects in wheat sown on sodic soils in India (Swarup & Sharma 1993). However, despite the importance of N fertiliser application in ameliorating the adverse effects of waterlogging, little or no research has been conducted to explore the potential of CRFs in mitigating the

effects of waterlogging. Most studies have focused on conventional N sources (Swarup & Sharma 1993), with no deliberate attempt to explore the potential CRFs. In this study, two experiments were conducted to understand the role of N fertiliser application in improving plant growth and development during and after waterlogging. The first experiment focused on assessing selected wheat and barley varieties for tolerance to prolonged waterlogging, on the hypothesis that waterlogging will significantly decrease the growth and development of all selected wheat and barley varieties. Following the identification of waterlogging intolerant wheat and barley varieties, a second experiment was conducted to assess their response to N fertiliser application with the hypothesis that CRF will significantly increase the growth and physiological responses of selected wheat and barley varieties than conventional split-applied urea.

3.3 Materials and methods

3.3.1 Location of the experiments

The two glasshouse studies were conducted at the Horticultural Research Centre (HRC) of the University of Tasmania, Sandy Bay campus located 42^0 91'S and 147^0 32'E. Air temperature inside the glasshouse was monitored using a data logger, Tinytag Ultra 2. Details of each experiment are given below.

3.3.2 Experiment 1

The experiment was conducted from January to April 2015. The glasshouse had an average minimum and maximum air temperatures of 12^0C and 33.0^0C, respectively and a relative humidity of 75%.

3.3.2.1 Crop management

A dark grey Vertosol soil obtained from Cressy Research and Demonstration Station 45 km south of Launceston was used as the growing medium. The site is located $41^0$72'S, $147^0$08'E and 150 m above sea level. It is also characterised by intermittent waterlogging (Pang et al. 2004). The soil was taken from the top 200 mm and sieved to remove rocks and fibrous root materials. A composite sample of the soil was analysed for nutrient composition (Table 3.1) by AgVita Analytical Pty Ltd. Pots of 0.15 m diameter and 0.15 m height were filled with 1.5 kg of soil and four wheat and barley seeds of the selected varieties noted below were sown per pot and later thinned to a single plant following emergence.

Table 3. 1: Soil nutrient composition for Experiment 1

Analyte	Result	Analyte	Result
Potassium (NH_4Cl)	1.28 (meq/100g)	pH (H_2O)	4.95
Calcium (NH_4Cl)	1.85 (meq/100g)	CECe	4.58 meq/100g
Magnesium (NH_4Cl)	0.75 (meq/100g)	EC	0.32 dS/m
Sodium (NH_4Cl)	0.70 (meq/100g)	Organic Carbon	1.63%
Aluminium (KCl)	0.42 (meq/100g)	Chloride	49.4 ppm
Copper (DTPA)	0.38 ppm	Oslen P	23.2 ppm
Iron (DTPA)	388.89 ppm	Nitrate (NO_3)	144.0 ppm
Zinc (DTPA)	4.91 ppm	Sulphur (MCP)	11.9 ppm
Manganese (DTPA)	54.38 ppm	Ca:NO_3 ratio	0.06
Calcium (%CEC)	40.41 %	Boron (hot water)	0.34 ppm
Magnesium (%CEC)	16.44 %	Nitrate (N-NO_3)	268.87 kg/ha
Potassium (%CEC)	27.90 %	Ammonium (N-NH_4)	47.12 kg/ha
Sodium (%CEC)	15.24 %		

3.3.2.2 Experimental design

The factorial experiment was designed as a split-plot with irrigation regime and variety as main-plot and subplot factors, respectively, and five replicates. The irrigation regime

included the control (well-drained) and waterlogged treatments. The control plants were watered four times per day for four minutes with an automated overhead sprinkler irrigation system while the waterlogged plants at the two leaf stage (GS12) were placed in vats (60 x 37 x 26 cm) and flooded with water. Waterlogging was instigated at once (same time for all plants) and the water level was maintained at 1 cm above the soil surface for the duration of the experiment (Colmer et al. 2001; Watson et al. 1976). The experiment was terminated at 70 days of waterlogging after complete plant senescence or significant crop damage had occurred. Crop varieties included: Brennan, Revenue, Mackellar, and Tennant for wheat and Naso Nijo and TX9425 for barley. The dual purpose winter wheat varieties suitable for grazing and grain production are tolerant to short-term waterlogging and perform well on a range of soil types particularly deep, well-drained soils. Ideally, the varieties are suited to the High Rainfall Zone and irrigated areas of Australia (Seednet 2018). For barley, Naso Nijo is a Japanese malting variety sensitive to waterlogging (Pang et al. 2004) and salinity (Gill et al. 2017) while TX9425 is a Chinese, two-rowed variety tolerant to waterlogging and salinity (Gill et al. 2017; Pang et al. 2004).

Agronomic practices such as weeding, pest and disease control were carried out to standard practice; pheromone traps were used to help control white flies and aphids. Osmocote Exact fertilizer was applied at a rate of 3 kg/m^3, 7.95 g/pot to prevent nutritional deficiencies.

3.3.2.3 Measurements

During the experiment, tiller number, number of green leaves, leaf chlorophyll content and number of dead leaves were recorded fortnightly. Leaf chlorophyll content was measured in the middle of the youngest fully expanded leaf using a chlorophyll meter (SPAD-502,

Osaka, Japan) (Hoel 1998). At the end of the experiment, the plants were harvested, oven dried at 60^0C for 48 hours and weighed to determine their above-ground DM (AGDM).

3.3.3 Experiment 2

The experiment was conducted from May to September 2015. The glasshouse had an average minimum and maximum air temperatures of 5.1^0C and 23.4^0C, respectively and a relative humidity of 80%. Additional lighting using incandescent light globes (20 watts) was introduced to maintain a photoperiod of 16 and 8 hours of light and darkness, respectively.

3.3.3.1 Crop management

A mixture of pure sand and sandy loam in a ratio of 4:1 was used as the growing medium. The sandy loam soil was obtained from the University Farm at Cambridge in the Barn paddock at a depth of 0-15 cm. The farm is located $42^0$84'S, $147^0$44'E and 51 m above sea level. A composite sample of the soil mixture was analysed for nutritional content by AgVita Analytical Pty Ltd (Table 3.2), the results were used to determine N fertiliser, macro- and micro- nutrient requirements. Wheat cv. Mackellar and barley cv. Naso Nijo seeds were sown in pots of 0.15 m diameter and 0.15 m height as described in Experiment 1. Mackellar and Naso Nijo were chosen because of their sensitivity to waterlogging based on the results of Experiment 1. Noteworthy, different soils were used for Experiments 1 and 2 because of the different aims of both experiments. In Experiment 1, a dark grey Vertosol soil obtained from Cressy Research and Demonstration Station was used because it is susceptible to waterlogging and the aim of the experiment was to assess the sensitivity of various wheat and barley varieties to waterlogging. However, the main objective of Experiment 2 was to assess how the selected waterlogging sensitive wheat and barley

varieties respond to N fertiliser application, which required use of a specific soil mix with low nitrogen content.

Table 3. 2: Soil mix nutrient composition for Experiment 2

Analyte	Result	Analyte	Result
Potassium (NH_4Cl)	0.30 (meq/100g)	pH (H_2O)	5.8
Calcium (NH_4Cl)	1.10 (meq/100g)	pH ($CaCl_2$)	4.93
Magnesium (NH_4Cl)	0.48 (meq/100g)	EC	0.07 dS/m
Sodium (NH_4Cl)	0.13 (meq/100g)	Organic Carbon	1.14%
Aluminium (KCl)	0.07 (meq/100g)	Chloride	36.1 ppm
Copper (DTPA)	0.19 ppm	Colwell P	32.9 ppm
Iron (DTPA)	80.00 ppm	Colwell K	125.4 ppm
Zinc (DTPA)	7.02 ppm	PBI	-
Manganese (DTPA)	3.97 ppm	Sulphur (KCl)	13.7 ppm
Calcium (%CEC)	54.70 %	Boron (hot water)	0.15 ppm
Magnesium (%CEC)	23.69 %	CECe	2.01 meq/100g
Potassium (%CEC)	14.98 %	Nitrate ($N\text{-}NO_3$)	8.19 kg/ha
Sodium (%CEC)	6.62 %	Ammonium ($N\text{-}NH_4$)	11.96 kg/ha
Total Carbon	1.36 %	Ca:NO_3 ratio	0.74
Total Nitrogen	0.13 %	Total Phosphorus	140.73 ppm

3.3.3.2 Experimental design

The experiment was a split-plot with irrigation regime and N source as the main-plot and subplot factors, respectively, and ten replicates. The irrigation regime included the control (well-drained) and waterlogged. Waterlogging was instigated at the two leaf stage (GS12) for 35 days. The procedure for waterlogging and maintenance of the control plants was as described in Experiment 1. However, similar N treatments were placed in the same vat to avoid lateral movement of N fertiliser through leaching. After waterlogging, half of the pots were used for destructive sampling and the remaining half were carefully removed from the vats and drained gradually to allow recovery for 35 days. Nitrogen source included: nil N, urea and CRF. The urea was split-applied in a ratio of 1:1 at sowing and at

one day after the termination of waterlogging (Matsunaga et al. 1994). The fertiliser was applied at a rate of 200 kg N/ha (Table 3.3) based on the nutrient analysis results in Table 3.2; urea (46-0-0) and CRF (39-0-0) were used. The CRF (Agrocote), currently known as Agromaster is polymer coated urea with a release period of three months. The fertiliser matches N release to the crop demand ensuring availability of N at critical growth stages through the advanced polymer coating that dictates N release into the soil (ImpactFertilisers 2018).

All pots were supplemented with P, K and other inadequate macro- and micro-nutrients. Phosphorous was applied at a rate of 25 kg/ha (0.46 g/pot) using Superphosphate fertilizer with 9.6% P. Potassium was applied at a rate of 200 kg/ha (0.71 g/pot) using muriate of potash with 50%K. Inadequate macronutrients (Ca and Mg) and micronutrients (Cu and B) were applied at rate of 150 g/m^2. With 6% Ca, 3% Mg, 1% Cu and 0.1% B in the Micromax fertilizer, each pot received 0.39g $CaCO_3$, 0.29g $Ca(OH)_2$, 0.39g $MgSO_4$, 0.1g $CuSO_4.5H_2O$ and 0.015g H_3BO_3. Agronomic practices such as weeding, pest and disease control were carried out according to standard practice; pheromone traps were used to prevent white flies and aphids.

Table 3. 3: Applied N treatments (kg fertiliser ha^{-1})

Nitrogen treatments	Basal ($kgNha^{-1}$)	g/pot	Top-dressed ($kgNha^{-1}$)	g/pot
Nil N	0	0	0	0
Urea (split-applied)	218	0.39	218	0.39
CRF	513	0.91	0	0

**Pot surface area: 0.0177 m^2*

3.3.3.3 Measurements

Both leaf chlorophyll content and chlorophyll fluorescence were recorded on a weekly basis. Leaf chlorophyll content was measured, as described in Experiment 1. Chlorophyll

fluorescence on the other hand, was measured using a Chlorophyll Fluorometer, OS-30p, Opti-Sciences, Inc fluorometer, USA at 10:00pm to allow optimum dark adaptation (Pang et al. 2007). The ratio of variable to maximum fluorescence was calculated according to Maxwell and Johnson (2000).

Destructive sampling was done after waterlogging and recovery. The samples were separated into dead leaf, green leaf and stem (tillers). Leaf area of the green leaves was determined using a Leaf Area meter (Li-3000C, John Morris Scientific Pty Ltd, LI-COR Biosciences). Tiller number was recorded and the green leaf, dead leaf and stem were oven dried at 40^0C for 48 hours and weighed to determine their DM and compute the AGDM. The roots were washed at harvest, oven-dried and weighed to determine the root DM. NUE was not determined because the plants were not seen through to maturity.

3.3.4 Data analysis

Data for non-destructive measurements were analysed using a repeated measures ANOVA in GenStat 17^{th} edition (GenStat 64-bit Release 17.1, VSN International Ltd). Two-way ANOVA was done for destructive measurements. Treatment means were deemed significant at 5% least significant difference (L.S.D).

3.4 Results

3.4.1 Experiment 1

3.4.1.1 Tiller number

Distinct differences between varieties were evident from 28 days of waterlogging (Figure 3.1). Mackellar, Naso Nijo and TX9425 were severely affected and while the tiller number for TX9425 improved slightly, Mackellar and Naso Nijo continued to decline. Overall,

tiller number was significantly affected by waterlogging (P = 0.001) (Figure 3.1). Mackellar and Tennant were most affected with an average of 86% and 62% reduction at 70 days after waterlogging was imposed, respectively. In contrast, Brennan and Revenue showed moderate tolerance to waterlogging, which reduced tiller number by an average of 40% and 26%, respectively. Barley varieties, Naso Nijo and TX9425 had relatively similar tiller numbers for both the control and waterlogged treatments. Wheat varieties, Brennan, Tennant and Revenue had more tillers than Naso Nijo and TX9425 for both the control and waterlogged treatments.

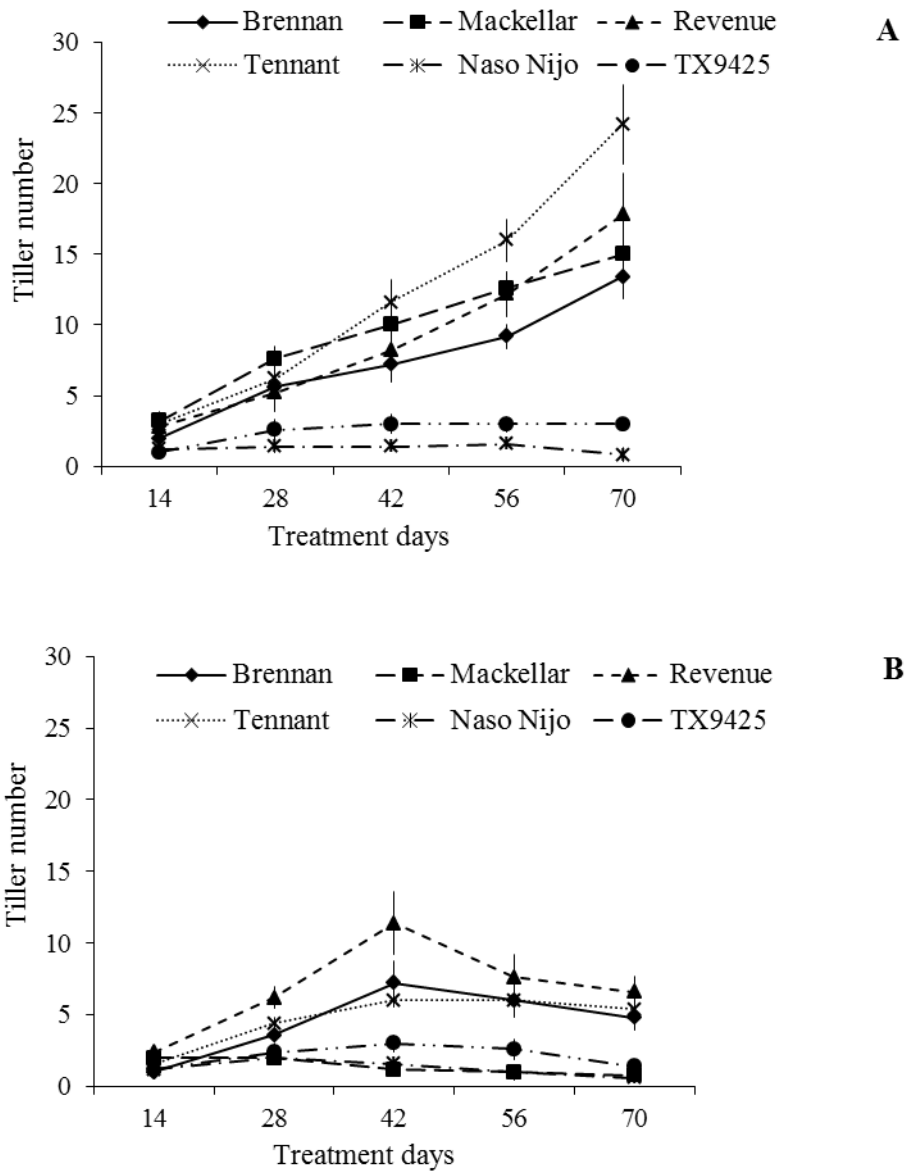

Figure 3.1: Tiller number of the different wheat and barley varieties for the control (A) and waterlogged (B) treatments. Bars indicate the s.e. (n = 5).

3.4.1.2 Number of green leaves

The number of green leaves was markedly reduced by waterlogging over time (Figure 3.2). A slight decline was observed at the start of waterlogging, however, at 28, 42 and 56 days of waterlogging, the number of green leaves was reduced by about 50% compared with the control plants. The decline increased slightly at 70 days of waterlogging. Wheat variety, Mackellar was the most affected compared with Revenue, Tennant and Brennan, which

had a significantly high number of green leaves at 70 days of waterlogging. Barley varieties, Naso Nijo and TX9425 had relatively the same number of green leaves for both the control and waterlogged treatments. They also had very few or no leaves left by the end of the experiment for both irrigation regimes. Wheat varieties, Brennan, Tennant and Revenue had more green leaves than both Naso Nijo and TX9425 for both the control and waterlogged treatments. Mackellar and Naso Nijo followed a similar trend throughout the duration of waterlogging (Figure 3.2).

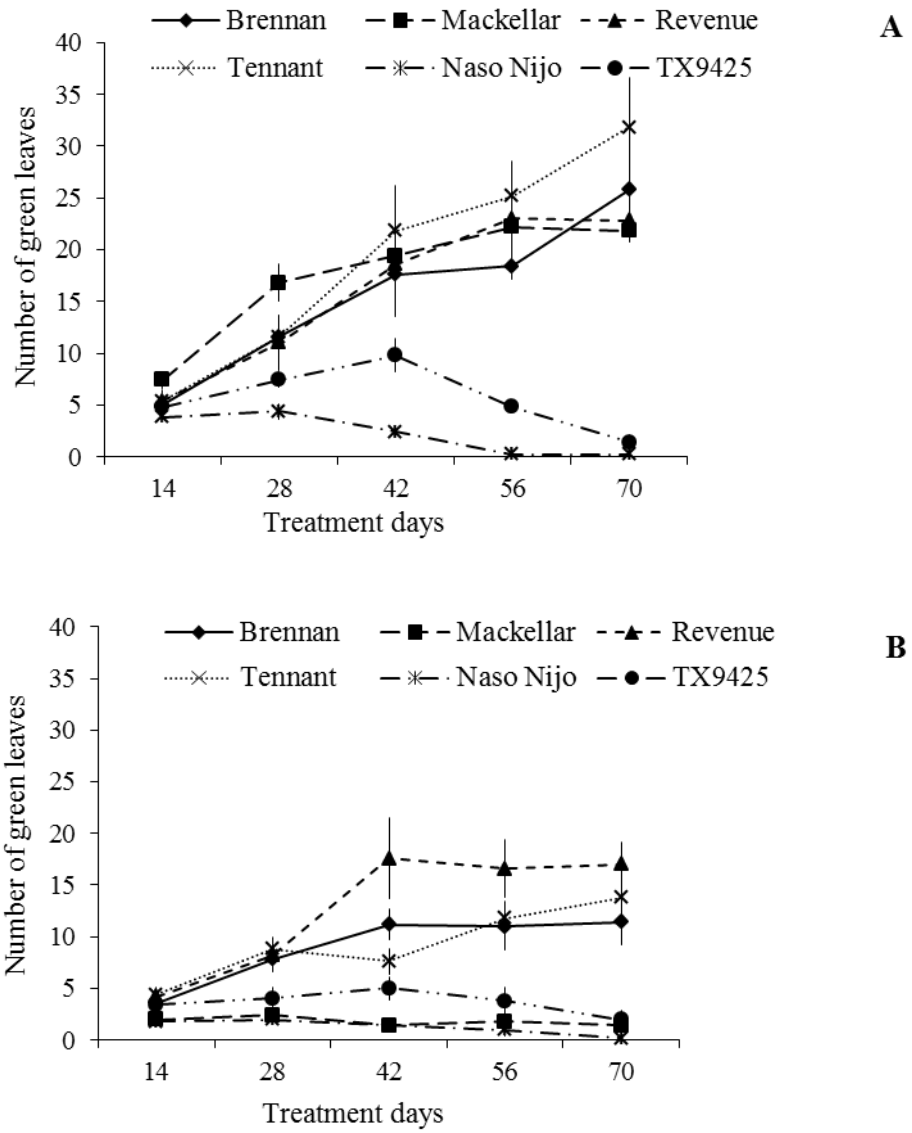

Figure 3.2: Number of green leaves for the different wheat and barley varieties for the control (A) and waterlogged (B) treatments. Bars indicate the s.e. (n = 5).

3.4.1.3 Leaf chlorophyll content

Waterlogging significantly ($P = 0.028$) decreased leaf chlorophyll content over time. Significant variations ($P = 0.001$) were also visible between the different varieties assessed (Figure 3.3). Naso Nijo and Mackellar had around 50% less chlorophyll content than TX9425, Brennan, Revenue and Tennant throughout the duration of waterlogging. Noteworthy, Naso Nijo had a steep decline in the leaf chlorophyll content of the control towards the end of the experiment as most leaves were chlorotic.

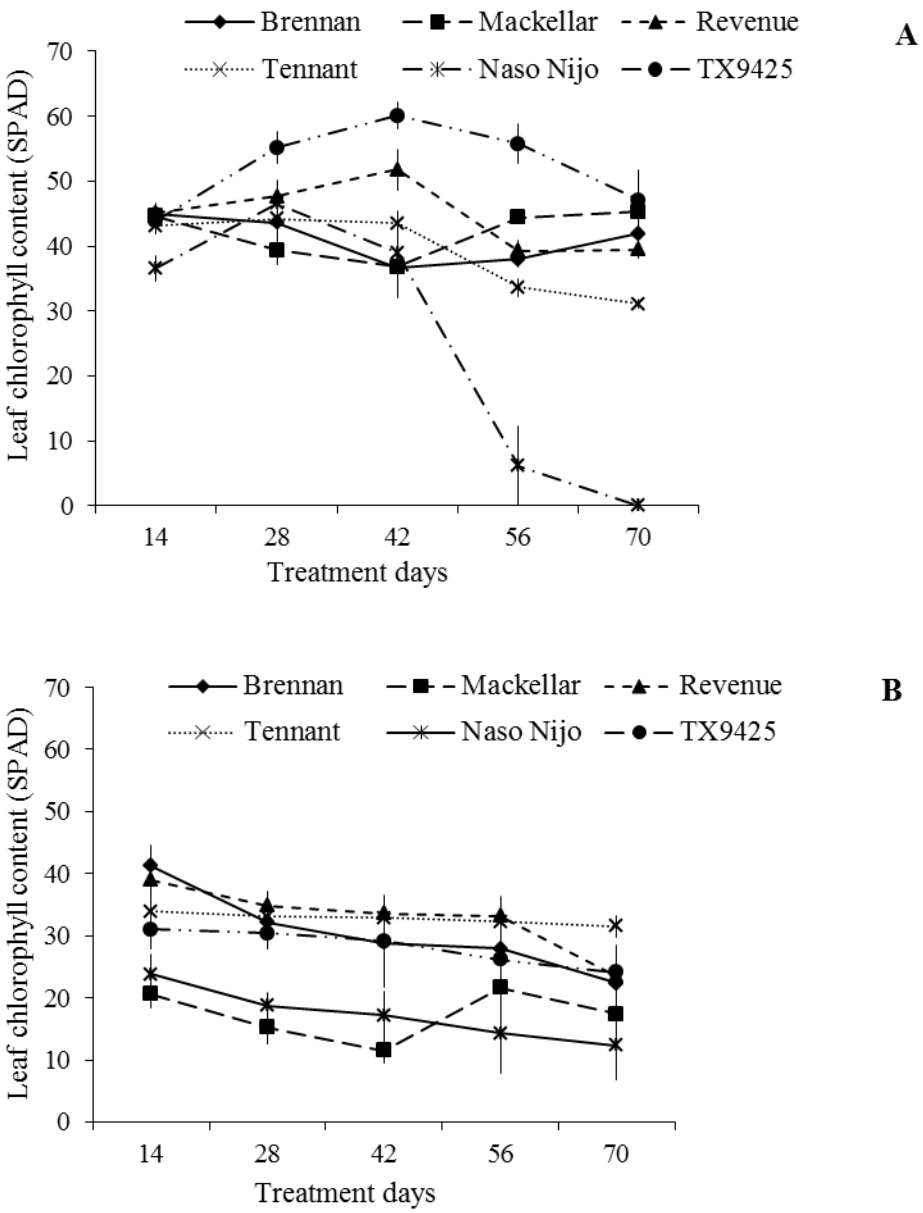

Figure 3.3: Leaf chlorophyll content for the different wheat and barley varieties for the control (A) and waterlogged (B) treatments. Bars indicate the s.e. (n = 5).

3.4.1.4 Number of dead leaves

The number of dead leaves increased gradually throughout the duration of the experiment (Figure 3.4). The process of leaf death was accelerated by waterlogging. For example, at 28 days of waterlogging, Mackellar, Naso Nijo and TX9425 had a significant number of

dead leaves than their respective controls. On the whole, wheat varieties had more dead leaves than barley.

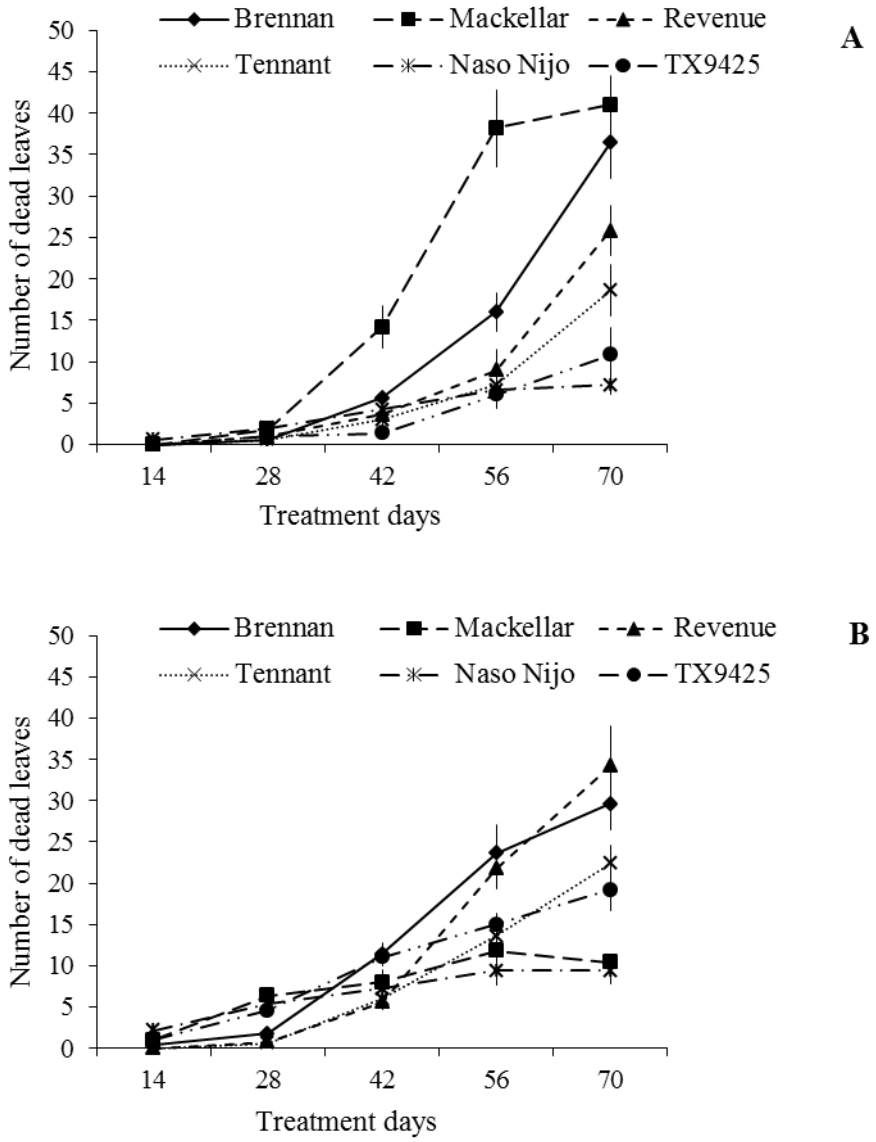

Figure 3.4: Number of dead leaves for the different wheat and barley varieties for the control (A) and waterlogged (B) treatments. Bars indicate the s.e. (n = 5).

3.4.1.5 Above-ground DM

The irrigation regime had a significant effect (P = 0.001) on the AGDM of all selected varieties. Significant differences (P = 0.001) were also evident among the different selected varieties. Mackellar and Naso Nijo were the most affected in contrast to Brennan,

Revenue, and Tennant, which showed moderate tolerance to waterlogging (Figure 3.5). Wheat varieties had more AGDM than barley varieties for both the control and waterlogged, with the exception of Mackellar. The results indicated that Mackellar and Naso Nijo were the most affected varieties by prolonged waterlogging.

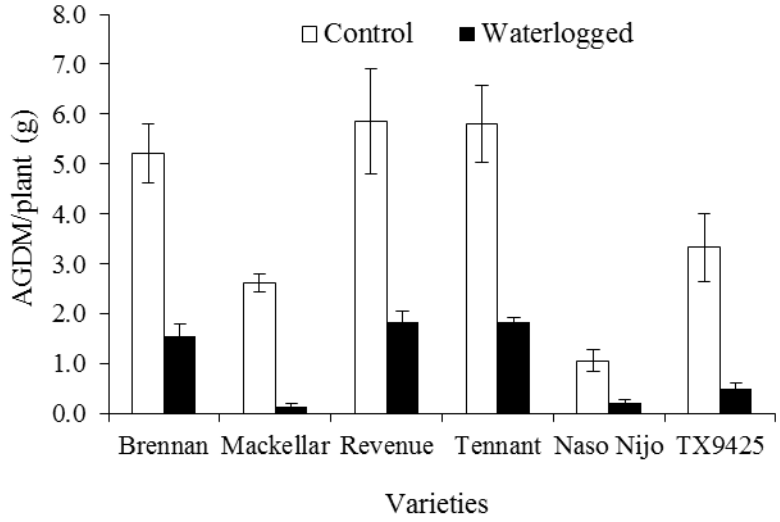

Figure 3.5: Above-ground DM of the different wheat and barley varieties at final harvest. Bars indicate the s.e. (n = 5).

3.4.2 Experiment 2

3.4.2.1 Leaf chlorophyll content

There was a significant interaction (P = 0.001) between irrigation regime and N fertiliser application over time for Mackellar during waterlogging (Figure 3.6). For Naso Nijo, the main treatments, irrigation regime and N fertiliser application had a significant effect on leaf chlorophyll content (P = 0.001) (Figure 3.7). Differences between N treatments for both varieties were evident as waterlogging progressed. The nil N treatments had the lowest leaf chlorophyll content compared with urea and CRF treatments for both Mackellar and Naso Nijo. Urea had a marked effect on leaf chlorophyll content at the start of waterlogging before declining gradually throughout the duration of waterlogging.

Interestingly, the urea of the control followed a similar trend too. The CRF on the other hand had a gradual positive effect on the leaf chlorophyll content of Mackellar and Naso Nijo. The leaf chlorophyll content of Mackellar was similar to that of the control, 49 SPAD units by the end of waterlogging.

During recovery, there was a significant interaction ($P < 0.05$) between irrigation regime and N fertiliser application over time for Mackellar and Naso Nijo (Figure 3.6 and 3.7). Differences between N treatments were evident for both Mackellar and Naso Nijo. Urea had a gradual rise in leaf chlorophyll content throughout recovery with the control peaking 56 SPAD units at 14 days while the previously waterlogged plants reached 52 SPAD units at 35 days in Mackellar. Naso Nijo followed a similar trend with plants reaching over 45 SPAD units at 35 days. Previously waterlogged plants treated with CRF had a rise in leaf chlorophyll content, which declined gradually towards the end of the experiment. Leaf chlorophyll fluorescence was also improved by N fertiliser application during waterlogging. The trends followed were relatively similar to those of leaf chlorophyll content for both Mackellar and Naso Nijo during waterlogging and recovery.

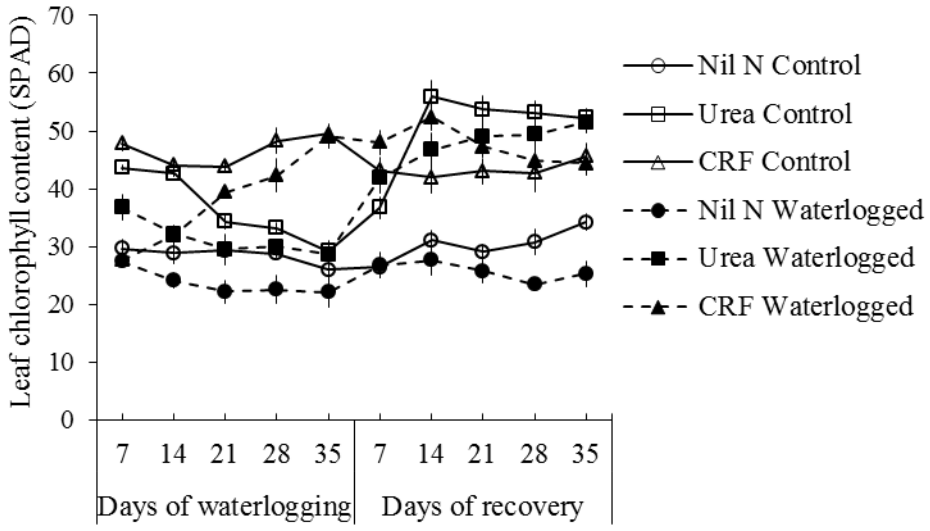

Figure 3.6: Leaf chlorophyll content of wheat cv. Mackellar under waterlogging and recovery. Bars indicate the s.e. (n = 5).

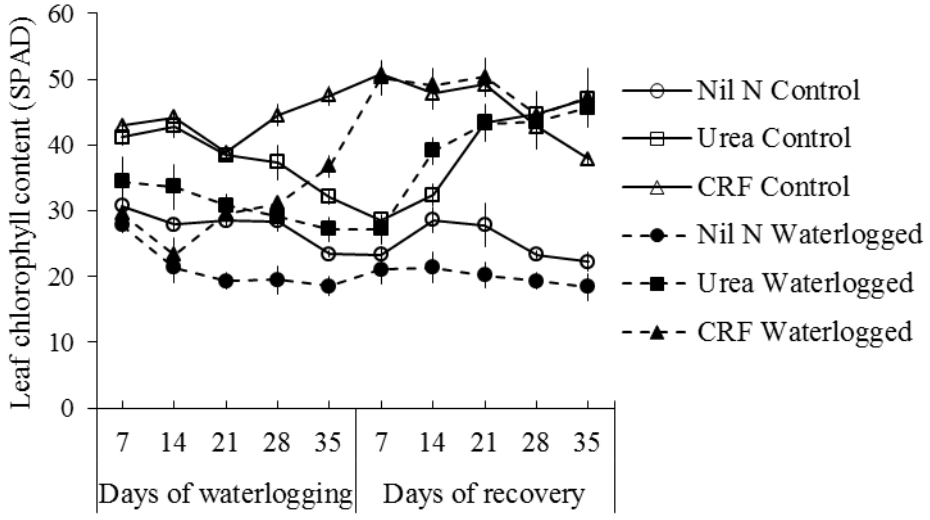

Figure 3.7: Leaf chlorophyll content of barley cv. Naso Nijo under waterlogging and recovery. Bars indicate the s.e. (n = 5).

3.4.2.2 Tiller number

There was a significant interaction (P = 0.001) between the irrigation regime and N fertiliser application for Naso Nijo during waterlogging and recovery. For Mackellar, N fertiliser application had a significant effect (P = 0.001) on tiller number during

waterlogging and recovery. Nitrogen fertiliser application improved tiller number for both Mackellar and Naso Nijo. The CRF had the highest number of tillers for both varieties during waterlogging (Figure 3.8A).

During recovery, N fertiliser significantly ($P = 0.001$) improved tiller number for both Mackellar and Naso Nijo (Figure 3.8B). The tiller number for Mackellar and Naso Nijo control plants increased significantly after top-dressing urea. For Mackellar, previously waterlogged plants had an average of eight tillers per plant compared with five tillers of the CRF treatment. For Naso Nijo however, CRF treated plants still had a higher number of tillers (5) compared with urea (3).

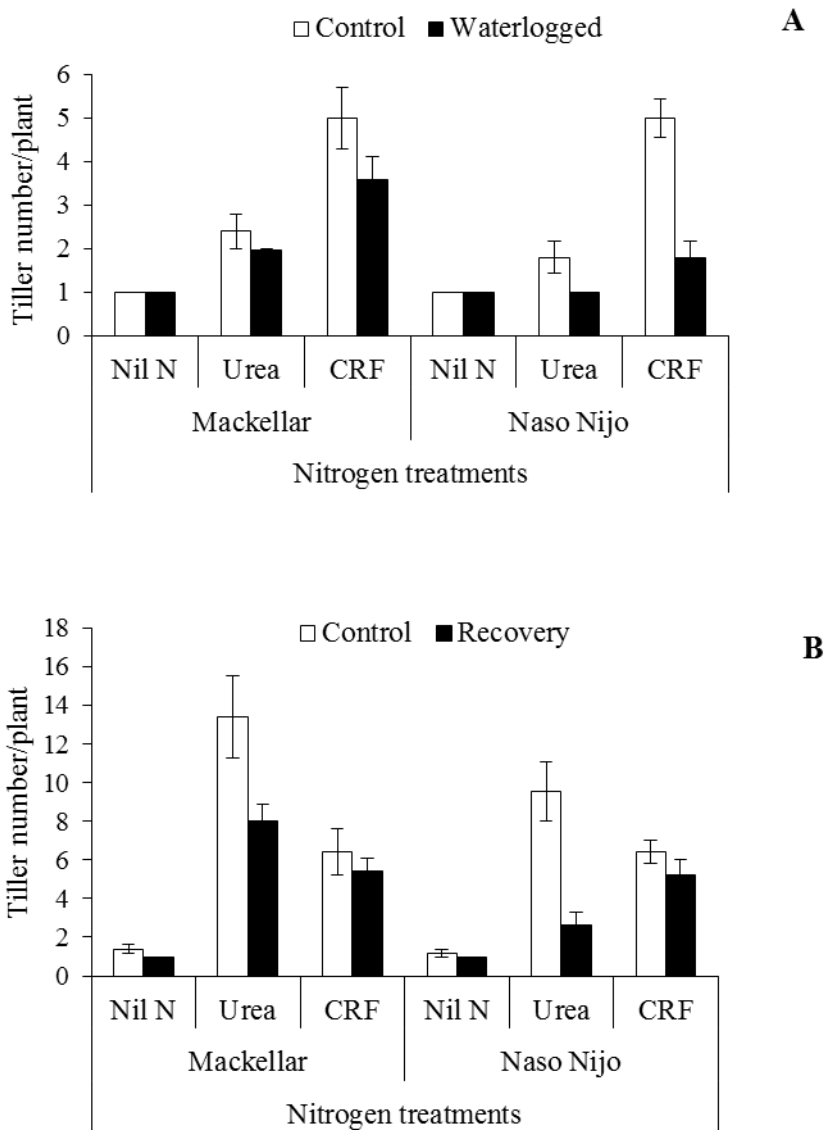

Figure 3.8: Tiller number of wheat cv. Mackellar and barley cv. Naso Nijo under waterlogging (A) and recovery (B). Bars indicate the s.e. (n = 5).

3.4.2.3 Green leaf area

There was a significant interaction (P = 0.001) between N fertiliser application and irrigation regime for both Mackellar and Naso Nijo during waterlogging. The green leaf area per plant was improved by N fertiliser application during waterlogging, with the CRF having the highest increment for both varieties followed by urea (Figure 3.9A). Mackellar was more responsive to CRF than Naso Nijo with 108 cm^2 in contrast with the 60 cm^2 of

Naso Nijo after waterlogging. The CRF significantly increased the green leaf area of the control plants with Mackellar and Naso Nijo having 240 cm^2 and 306 cm^2 of green leaf area, respectively compared with 87 cm^2 and 69 cm^2 of their respective urea treatments.

During recovery, N fertiliser application had a significant effect ($P = 0.001$) on green leaf area for Mackellar. There were no significant differences ($P = 0.862$) between the control and previously waterlogged Mackellar plants (Figure 3.9B). Top-dressing urea improved the green leaf area of Mackellar under recovery with urea having the highest amount of green leaf area, 399 cm^2, CRF and nil N had 267 cm^2 and 30 cm^2, respectively. For Naso Nijo, there was a significant interaction ($P = 0.001$) between N fertiliser application and irrigation regime. Improvement in the green leaf area of the urea treatment for the control was also observed.

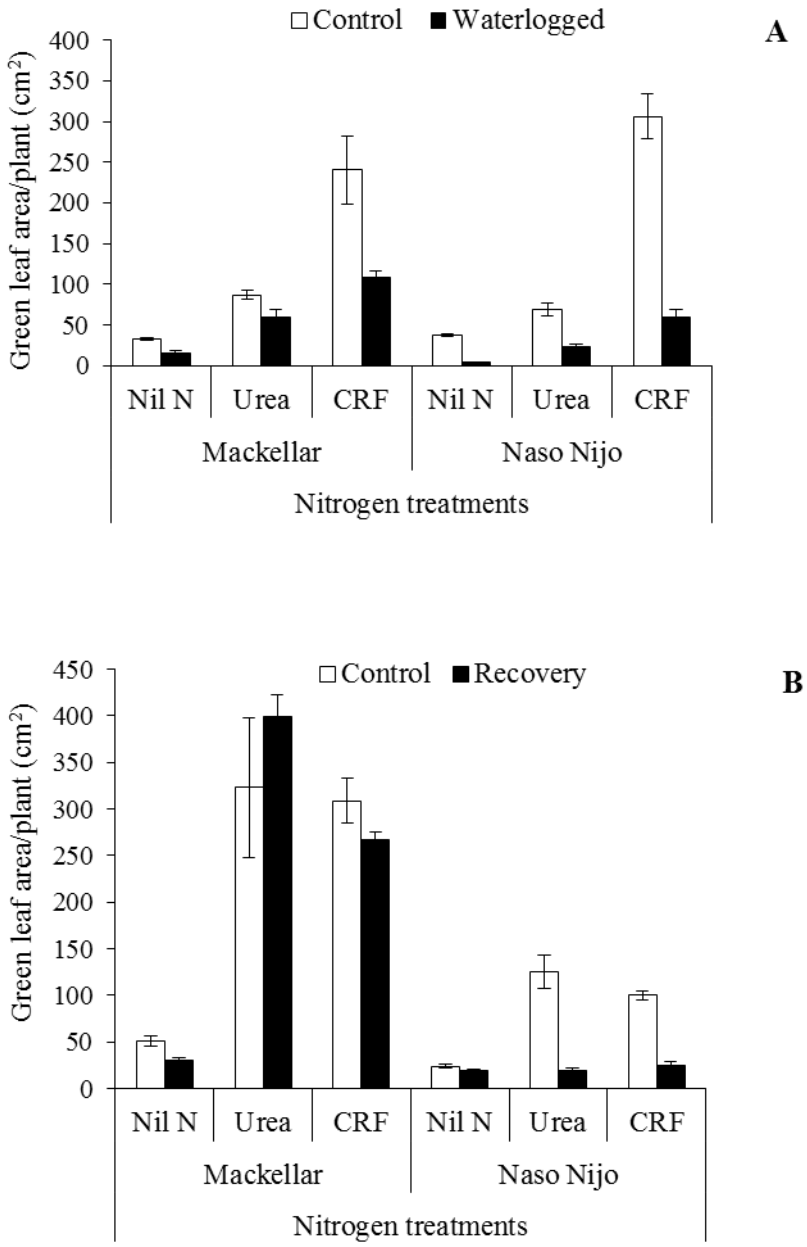

Figure 3.9: Green leaf area of wheat cv. Mackellar and barley cv. Naso Nijo under waterlogging (A) and recovery (B). Bars indicate the s.e. (n = 5).

3.4.2.4 Above-ground DM

During waterlogging, there was significant interaction ($P < 0.05$) between irrigation regime and N fertiliser application for both Mackellar and Naso Nijjo. The above-ground DM per plant was improved by N fertiliser application for both Mackellar and Naso Nijo (Figure 3.10A). The CRF had the highest amount of AGDM for both Mackellar and Naso

Nijo, though not significantly different from urea. For the control, the CRF significantly increased AGDM of both Mackellar and Naso Nijo.

After recovery, there was significant interaction ($P = 0.001$) between irrigation regime and N fertiliser application for Mackellar while the main treatments, irrigation regime and N fertiliser application had a significant effect ($P = 0.001$) on the AGDM of Naso Nijo. Nitrogen fertiliser improved AGDM for both the control and previously waterlogged plants of Mackellar and Naso Nijo (Figure 3.10B). Top-dressing urea after waterlogging significantly improved the AGDM of Mackellar (5 g) compared with Naso Nijo (2 g). However, for the control, CRF still had the highest amount of AGDM for both Mackellar and Naso Nijo.

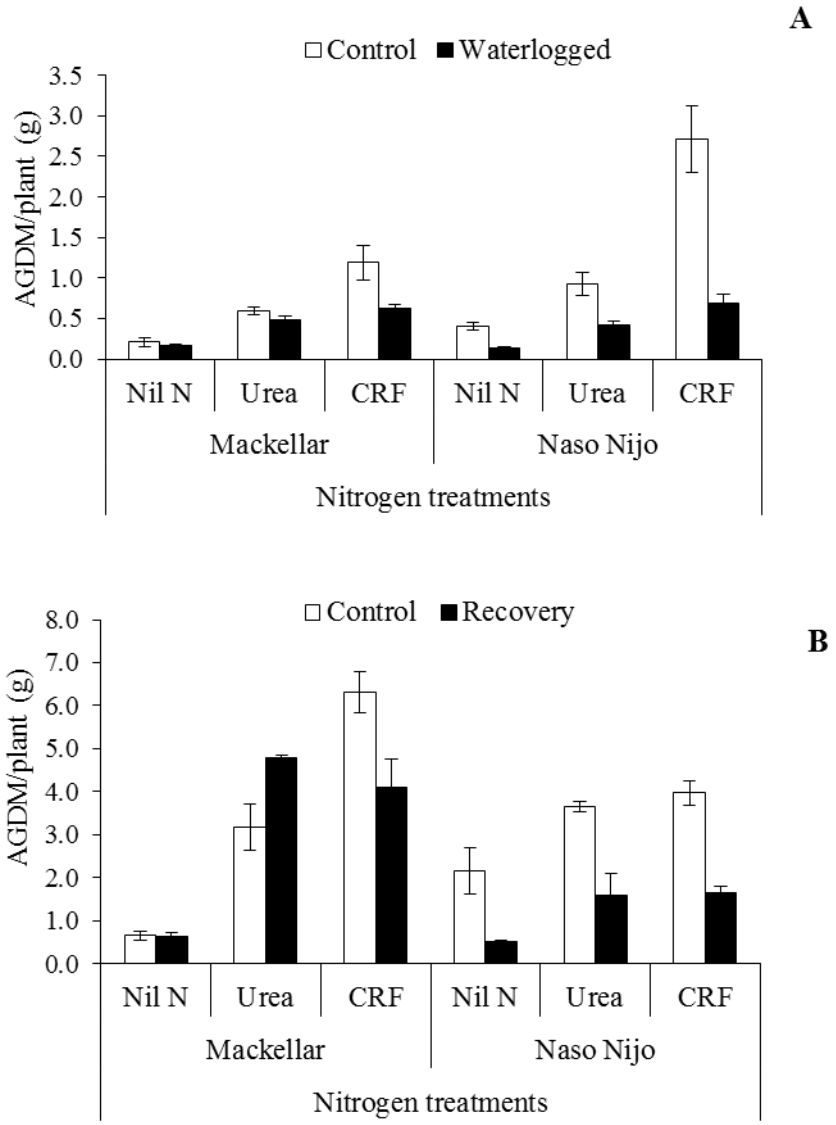

Figure 3.10: Above-ground DM of wheat cv. Mackellar and barley cv. Naso Nijo under waterlogging (A) and recovery (B). Bars indicate the s.e. (n = 5).

3.4.2.5 Root DM

There was an interaction between irrigation regime, N fertiliser application and variety. For Naso Nijo, the interaction was significant (P = 0.001) between irrigation regime and N fertiliser application during waterlogging and recovery. For Mackellar, the irrigation regime and N fertiliser application had a significant effect (P = 0.001) on root DM during

waterlogging. During recovery there was a significant interaction ($P = 0.002$) between the two factors. Nitrogen treatments with applied N generally had higher root DM than nil N for both irrigation regimes (Figure 3.11A and 3.11B). Urea and CRF had 0.16 g and 0.08 g, respectively compared with 0.04 g of nil N for Mackellar after waterlogging. Similarly, for Naso Nijo, urea and CRF had 0.03 g and 0.04 g, respectively compared with 0.01 g of nil N. The control plants with N fertiliser had significantly higher root DM than nil N treatments and corresponding waterlogged treatments after waterlogging and recovery (Figure 3.11A and 3.11B). Over all, there was an increase in the root DM of both Mackellar and Naso Nijo after recovery with treatments with applied N registering the highest increase.

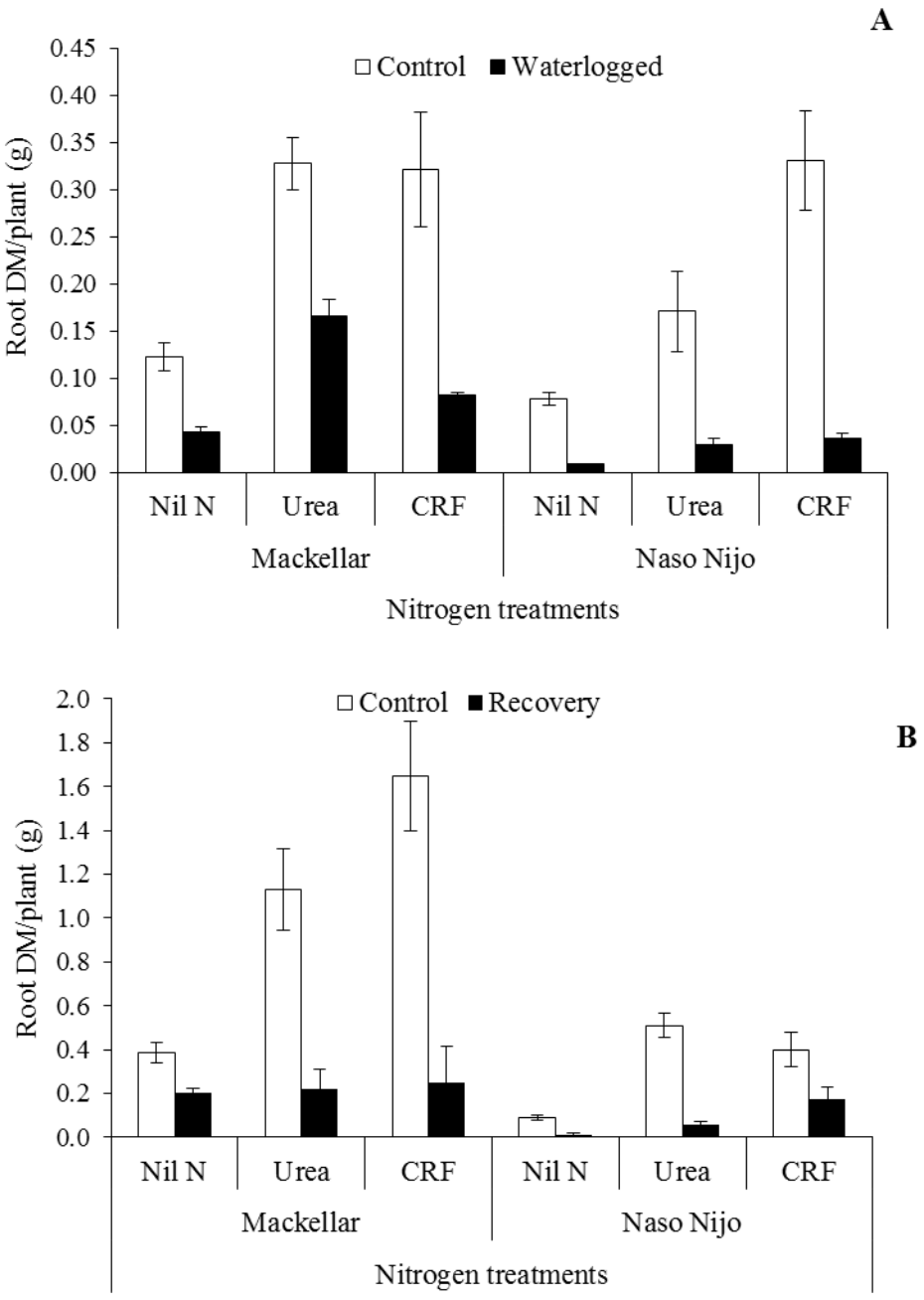

Figure 3.11: Root DM of wheat cv. Mackellar and barley cv. Naso Nijo under waterlogging. Bars indicate the s.e. (n = 5).

3.5 Discussion

Results from the present study, and those of Malik et al. (2002); Pang et al.(2007); Pang et al. (2004), show that waterlogging decreases plant growth and development. This reduction in plant growth is attributed to the limited availability and uptake of essential plant nutrients (Huang et al. 1994b; Pang et al. 2007; Pang et al. 2004). Nutrient availability is reduced due to substantial dilution of nutrient concentrations and leaching of mobile nutrients; reduced nutrient uptake is attributed to restricted root growth and death during waterlogging (Condon & Giunta 2003). Waterlogging also causes early onset of leaf and tiller senescence (Collaku & Harrison 2005; Hossain & Uddin 2011) causing a decline in leaf chlorophyll content, leaf and tiller number. Variations in susceptibility to waterlogging between and within wheat or barley varieties observed in this study were also highlighted by Setter et al. (1999) and Thomson et al. (1992). Similarly, Zhou (2010), discriminates that wheat is often more tolerant to waterlogging than barley. This was the case for this study particularly wheat varieties, Brennan, Revenue and Tennant were more tolerant to waterlogging than barley varieties, TX9425 and Naso Nijo. The sensitivity of Naso Nijo to waterlogging compared with TX9425 has been documented in a study by Pang et al. (2004). Tolerance to waterlogging is also attributed to the ability of plants to modify their root architecture to maximize resource capture (Bailey-Serres & Voesenek 2008). For instance, the development of aerenchyma to facilitate long-distance gas transport between the aerobic shoot and the anaerobic root (Drew 1988; Wenger 2010), the development of numerous adventitious roots at the shoot base (Colmer et al. 2001; Pang et al. 2007; Pang et al. 2004; Sairam et al. 2008) and elongation of internodes as an escape strategy (Bailey-Serres & Voesenek 2008; Parelle et al. 2010; Vartapetian & Jackson 1997).

Previous researchers reported similar findings with respect to N fertiliser application improving plant growth under waterlogged conditions (Pang et al. 2007; Robertson et al. 2009; Swarup & Sharma 1993). This improvement could be attributed to the significant role played by N in improving the efficiency of the photosynthetic apparatus particularly through the synthesis of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), which facilitates CO_2 assimilation (Hirel et al. 2007; Shangguan et al. 2000a; Shangguan et al. 2000b). The study results also showed that CRFs can improve plant growth under waterlogged conditions. Indeed, the potential of foliar N sprays and use of conventional N sources particularly urea in alleviating the adverse effects of waterlogging has been explored (Pang et al. 2007; Robertson et al. 2009; Swarup & Sharma 1993). CRFs can sustain N supply throughout the crop growth period by synchronising N supply with crop demand (Trenkel 2010). Agrocote through its advanced polymer coating matches N release into the soil to the crop demand, ensuring availability of N at critical growth stages and throughout the crop growth duration (ImpactFertilisers 2018). This reduces N loss and increases N assimilation by more than 50% according to Lubkowski and Grzmil (2007), with resultant increase in crop productivity. Despite the solubility of urea and ability to readily provide N for plant uptake, assimilation and utilisation, it is susceptible to leaching, denitrification and volatilization (Rane et al. 2011), which decreases its efficiency during waterlogging. Often applied after waterlogging; foliar N sprays and top-dressed urea can compensate either fully or partially for the reduction in cereal growth caused by waterlogging (Matsunaga et al. 1994; Watson et al. 1976). Moreover, the basal urea applied at sowing enhances vegetative growth particularly tillering and canopy size and duration (Cai et al. 2012; Fois et al. 2009), which helps plants to offset the adverse effects of waterlogging. Furthermore, in agreement with previous studies by Pang et al. (2004), the study results indicated that the recovery period enhances the production of secondary

tillers (Tan et al. 2008) and plant growth rates (Colmer et al. 2001). This suggests that during recovery, plants produce new adventitious roots with aerenchyma tissue (Huang et al. 1994a; Huang et al. 1994b; Pang et al. 2004), and there is regrowth of existing roots, which increases root biomass and plant nutrient uptake rates.

Despite the potential of N fertiliser application in ameliorating the adverse effects of waterlogging (Pang et al. 2007), no research has been conducted to explore the potential of CRFs in mitigating the effects of waterlogging. The study findings showed that the CRF can sustain N supply throughout the duration of the experiment, a feature attributed to its biochemical properties. The study also showed that appropriate timing of N fertiliser application equally important and top-dressing urea after waterlogging can help plants compensate for the adverse effects of waterlogging, which did not support the proposed hypothesis of CRF significantly increasing the growth and physiological responses of selected wheat and barley varieties than conventional split-applied urea.

3.6 Conclusion

Waterlogging decreased plant growth and development. Mackellar and Naso Nijo were the most sensitive varieties to waterlogging for both wheat and barley, respectively. Varieties: Brennan, Revenue, Tennant and TX9425 showed moderate tolerance to waterlogging. Nitrogen fertiliser application improved wheat cv. Mackellar and barley cv. Naso Nijo growth during waterlogging and recovery. The CRF sustained N supply throughout the duration of the experiment; conventional urea readily provided N at the commencement of waterlogging. Top-dressing urea after waterlogging and the termination of waterlogging to allow recovery enhances plant growth and development. This highlights the potential of enhanced-efficiency fertilisers such as CRFs in improving plant growth under waterlogged conditions. It also shows how timely conventional urea application can help ameliorate the

adverse effects of waterlogging. Basing on these findings, the next chapter seeks to explore how CRF compares with conventional urea in improving wheat (cv. Revenue) growth and NUE under waterlogged field conditions. Revenue is one of the currently recommended varieties for wheat growth in Tasmania.

Chapter Four: Effect of soil moisture availability and nitrogen source on wheat growth and nitrogen-use efficiency

4.1 Abstract

Soil moisture content has a significant impact on nutrient availability for plant growth and development. Excessive soil moisture due to waterlogging can severely reduce nutrient availability through substantial dilution of nutrient concentrations and leaching of mobile nutrients like nitrogen (N). This decreases N uptake and utilization by plants hence reducing nitrogen-use efficiency (NUE). Nitrogen-use efficiency can be improved by using controlled-release fertilisers (CRFs) and appropriate timing of N fertiliser application. This study investigated whether timing of N application and source of applied N can alleviate the adverse effects of waterlogging on wheat growth and improve NUE. The experiment conducted at Cressy Research and Demonstration Station from June 2014 to January 2015 was designed as a split-plot with irrigation regime and N fertiliser application as main-plots and subplots, respectively, with three replicates. The irrigation regime included: rainfed, irrigated and waterlogged while N fertiliser application had nil N, single-applied urea, split-applied urea and CRF treatments. Wheat growth and yield attributes including leaf area, leaf area index (LAI), tiller number, ear number, grain yield, above-ground dry matter (AGDM), thousand grain weight (TGW) and harvest index (HI) were determined at specific growth stages namely: stem elongation (GS32), anthesis (GS61) and maturity (GS92). NUE, its components, N harvest index (NHI) and grain protein content (GPC) were also determined. The study showed a significant interaction between irrigation regime and N fertiliser application for tiller number (P = 0.013) and leaf area (P = 0.014) at GS32. The AGDM per plant was significantly affected by irrigation regime (P = 0.001)

and N fertiliser application ($P = 0.012$) while AGDM per unit area was significantly affected by the irrigation regime only ($P = 0.001$). At GS61, the irrigation regime ($P = 0.001$) and N fertiliser application ($P < 0.05$) had a significant effect on the tiller number, ear number and AGDM per plant. There was no significant interaction between the irrigation regime and N fertiliser application ($P > 0.05$). At maturity, yield attributes including tiller number, ear number and grain yield were significantly affected by irrigation regime ($P = 0.001$) and N fertiliser application ($P < 0.05$). The CRF had the highest grain yield for all irrigation regimes with 9.2 t/ha, 9.4 t/ha and 6.8 t/ha for the rainfed, irrigated and waterlogged, respectively. The urea treatments had an average of 8 t/ha for both the rainfed and irrigated, and 5.3 t/ha for the waterlogged. NUE was significantly affected by irrigation regime ($P = 0.001$) and N fertiliser application ($P = 0.036$). The waterlogged plants had the lowest NUE for all corresponding N treatments under the rainfed and irrigated regimes. The CRF had the highest NUE for all irrigation regimes. Under waterlogged conditions, the CRF improved NUE by 17% and 27% more than single- and split- applied urea, respectively. No significant variations were observed between N treatments for components of NUE, NHI or GPC for the rainfed and waterlogged treatments.

Key words: duplex soils; enhanced-efficiency fertilisers; yield components; nitrogen fertilisers; *Triticum aestivum* L.; high rainfall zone.

4.2 Introduction

Although water availability plays a significant role in crop growth development (Sadras & Angus 2006), excessive soil moisture content can cause detrimental effects to plant growth (Zhang et al. 2006; Zhang et al. 2004). In Tasmania an estimated 23% of the soils are duplex and susceptible to waterlogging (Cotching et al. 2009), causing wheat grain yield

losses of *ca*. 30-50% (Zhou 2010). These yield losses are usually as a result of restricted root growth, reduced tiller number, premature leaf senescence and production of sterile florets (Hossain & Uddin 2011).

Nitrogen fertiliser application plays a significant role in improving plant growth and development under waterlogged conditions. Various studies have shown that N, applied as foliar sprays or top-dressed, can increase grain yield through a combination of root and shoot growth including plant height, tiller number and spikelet number (Pang et al. 2007; Robertson et al. 2009; Swarup & Sharma 1993). For instance, Robertson et al. (2009) showed that N fertiliser applied after waterlogging can increase grain yield by ~20%. Nonetheless, conventional N fertilisers are easily lost through leaching, denitrification, immobilization, volatilization and surface runoff during waterlogging (Ju et al. 2009; Lubkowski & Grzmil 2007; Mathers et al. 2007; Shaviv & Mikkelsen 1993), all of which can reduce nitrogen-use efficiency (NUE). With the increasing environmental (Mathers et al. 2007) and health concerns (Ju et al. 2009) associated with N fertiliser use in agriculture, there is need to maximize NUE. Timing of N application influences N loss and split N application is often recommended over single N application (Cai et al. 2011). Controlled-release fertilisers (CRFs) often referred to as enhanced-efficiency fertilisers have been reported to be a viable option for improving NUE through synchronisation between N supply and crop demand (Chen et al. 2008). However, most work under waterlogged conditions has focused on the conventional N sources such as urea with no deliberate intention to explore the potential of CRFs. Furthermore, most N is top-dressed after waterlogging, which can be difficult under field conditions as the soil moisture content is often too high for efficient mechanization. The evaluation of the potential of N fertiliser application in reducing the adverse effects of waterlogging under field conditions has received little attention.

This study investigated whether timing of N application and source of applied N can alleviate the adverse effects of waterlogging on wheat growth and improve NUE. The study evaluated wheat growth responses and yield components under different irrigation regimes and nitrogen treatments and quantified NUE, its components and grain protein content (GPC) under different irrigation regimes. It was hypothesised that under similar irrigation management, the CRF will significantly increase wheat growth attributes, yield components, NUE, NUE components, NHI and GPC compared with the single- and split-applied urea treatments.

4.3 Materials and methods

The study was conducted at Cressy Research and Demonstration Station 45 km south of Launceston, Tasmania. The site is located 41.73^0S, 147.08^0E and 148 m above sea level (BOM 2015). The site soil is duplex in nature posing significant problems to agricultural use (Grose & Moreton 1996). The area receives an average annual rainfall of 626 mm, making it one of the high rainfall zones for grain production in Australia. However, the sinuous nature of the drainage pattern and flat topography make the area prone to waterlogging particularly during autumn and winter (Hardie et al. 2012).

A split-plot design with irrigation regime (rainfed, irrigated and waterlogged) and N application (nil N, single-applied urea, split-applied urea and CRF, poly-S coated agrocote) as the main-plot and subplot factors with three replicates was used. The rainfed treatment received 352 mm of rain during the experimental period (Figure 4.1) (BOM 2015) whilst the irrigated block in addition to the natural rainfall, received 25 mm of water twice a week from sowing until the mid-grain filling phase using an overhead centre pivot system. Waterlogging was instigated at tillering (GS20) for 28 days using a drip irrigation system setup to ensure the water level is kept above the soil surface (Collaku & Harrison

2005; Yaduvanshi et al. 2012). Nitrogen fertiliser was applied at a rate of 90 kg N/ha based on the nutrient analysis results in Table 4.1. Nutrient analysis for each composite soil sample at different soil depths (0-15 cm, 15-30 cm, 30-60 cm and 60-90 cm) was carried out by AgVita Analytical Pty Ltd. Urea (46-0-0) and CRF (39-0-0) were used. Single-applied urea and CRF had full amounts applied once at sowing while split-applied urea had 40% applied at sowing and the remaining 60% top-dressed at GS32 after waterlogging (Collaku & Harrison 2005).

Table 4. 1: Experimental site soil nutrient composition

Analyte	Soil depth			
	0-15 cm	15-30 cm	30-60 cm	60-90 cm
Potassium (NH_4Cl)	0.34 (meq/100g)	0.25 (meq/100g)	0.18 (meq/100g)	0.14 (meq/100g)
Calcium (NH_4Cl)	2.09 (meq/100g)	1.63 (meq/100g)	1.65 (meq/100g)	1.31 (meq/100g)
Magnesium (NH_4Cl)	0.58 (meq/100g)	0.45 (meq/100g)	0.41 (meq/100g)	0.41 (meq/100g)
Sodium (NH_4Cl)	0.13 (meq/100g)	0.11 (meq/100g)	0.11 (meq/100g)	0.13 (meq/100g)
Aluminium (KCl)	0.56 (meq/100g)	0.63 (meq/100g)	0.47 (meq/100g)	0.13 (meq/100g)
Copper (DTPA)	0.34 ppm	0.34 ppm	0.27 ppm	0.22 ppm
Iron (DTPA)	418.11 ppm	401.52 ppm	232.93 ppm	136.51 ppm
Zinc (DTPA)	1.60 ppm	1.32 ppm	0.73 ppm	0.39 ppm
Manganese (DTPA)	40.41 ppm	33.19 ppm	17.59 ppm	15.69 ppm
Calcium (%CEC)	66.59%	66.82%	69.94%	65.83%
Magnesium (%CEC)	18.56%	18.39%	17.47%	20.69%
Potassium (%CEC)	10.73%	10.16%	7.78%	7.06%
Sodium (%CEC)	4.12%	4.63%	4.80%	6.41%
Total Carbon	1.86%	1.60%	4.36%	0.47%
Total Nitrogen	0.13%	0.14%	0.32%	0.004%
Ca: Mg ratio	3.59	3.63	4.00	3.18
pH (H_2O)	5.19	5.14	5.4	5.8
pH ($CaCl_2$)	4.6	4.56	4.68	4.93
EC	0.07 dS/m	0.07 dS/m	0.04 dS/m	0.03 dS/m
Organic Carbon	2.73%	2.29%	1.78%	1.14%
Chloride	17.40 ppm	22.80 ppm	17.20 ppm	8.85 ppm
Colwell P	53.30 ppm	52.70 ppm	27.70 ppm	14.20 ppm
Colwell K	433.87 ppm	322.97 ppm	370.17 ppm	539.10 ppm
PBI	74.65 units	62.11 units	45.51 units	44.52 units
Sulphur (KCl)	8.17 ppm	7.92 ppm	7.79 ppm	8.81 ppm
Boron (hot water)	0.44 ppm	0.33 ppm	0.26 ppm	0.22 ppm
CECe	3.13 meq/100g	2.44 meq/100g	2.35 meq/100g	1.99 meq/100g
Nitrate ($N\text{-}NO_3$)	19.1 kg/ha	24.7 kg/ha	29.7 kg/ha	17.2 kg/ha
Ammonium ($N\text{-}NH_4$)	4.5 kg/ha	3.0 kg/ha	3.6 kg/ha	2.9 kg/ha
Ca:NO_3 ratio	0.63	0.46	0.77	1.10
Total Phosphorus	372.82 ppm	331.48 ppm	215.92 ppm	162.86 ppm
C:N ratio	14.88	11.87	13.85	111.69

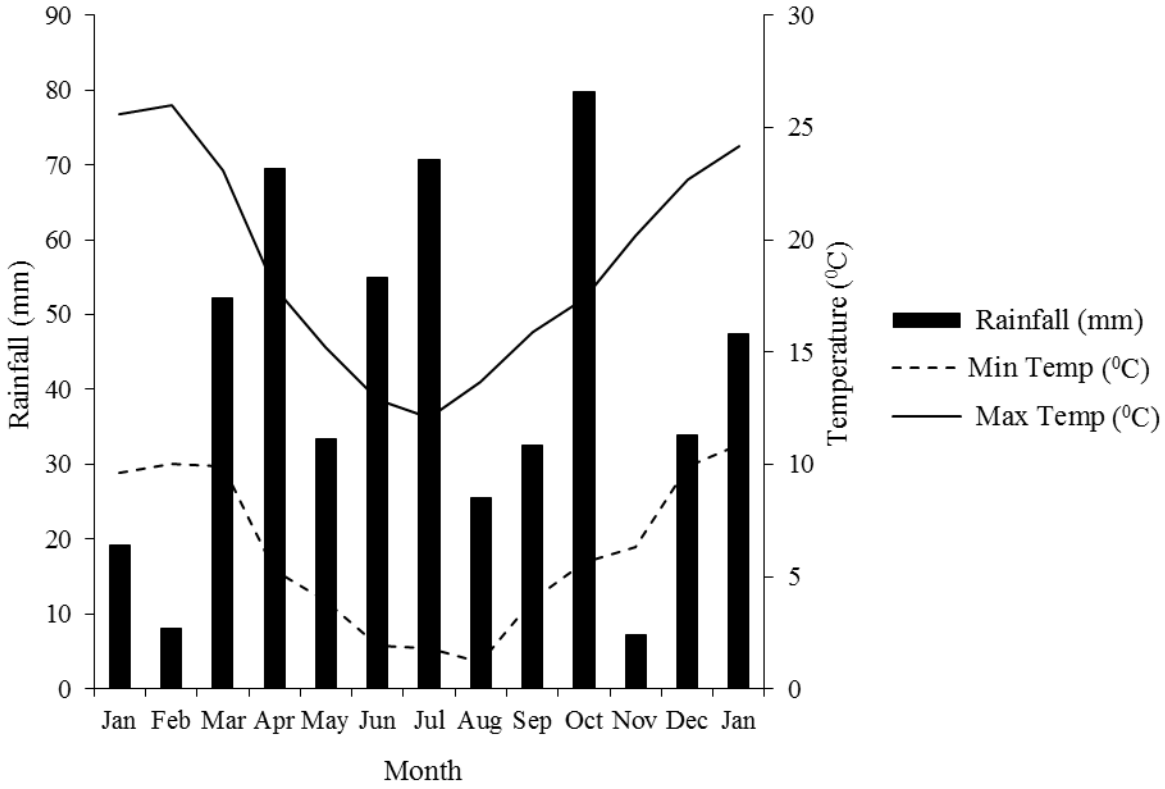

Figure 4.1: Rainfall and temperature at Cressy Research and Demonstration Station during the experimental period 2014/2015.

Prior to sowing, the rainfed and irrigated blocks were formed into 12 raised beds each using a commercial bed former with a depth of 300 mm and a furrow width of 300 mm. The waterlogged block consisted of 12 flat (unbedded) plots. Wheat (*Triticum aestivum*) cv. Revenue dressed with fungicide "Real" (Triticonazole and Cypermethrin) was sown on the 6th of June 2014 using a Cone Seeder with a target plant population of 220 plants per m^2 and a field factor of 15%. The crop with an expected seedling emergence of 85% was drilled at a depth of 30 mm and a seed rate of 125 kg/ha with 150 mm row spacing in plots 8 m long and 1.8 m wide. At sowing, a starter fertiliser with no nitrogen (N: P: K: S: Ca; 0-6-17-7-13) was applied at a rate of 250 kg/ha. During waterlogging, the rise in the depth of the water table was monitored using small diameter (50 mm) PVC tubes (piezometers) installed to a depth of 1 m (Sundaram et al. 2009). Three piezometer tubes were installed in the rainfed, irrigated and waterlogged blocks at 6 m spacing. The depth to the water

table was recorded manually using a sampler and tape measure. The depth of the water table provides a useful indication as to whether the soil is aerobic or anaerobic (Setter & Waters 2003). In this study, the depth to the water table was maintained within the top 300 mm of the rhizosphere for the waterlogged treatment. A depth of 300 mm is positively correlated with crop yields on duplex soils (Cox & McFarlane 1995) and is described as the primary root zone (Setter & Waters 2003).

Plants were monitored for specific growth stages (Zadoks et al. 1974). At the start of stem elongation (GS32) plants within a 0.3 m^2 quadrat were hand harvested in each plot, 12 plants were randomly selected and processed for tiller number, green leaf area and above-ground dry matter (AGDM) per plant. The remaining plants were bulked, oven dried at 60^0C for 48 hours and weighed to determine the AGDM per unit area. The green leaf area was determined using a portable Leaf Area meter (LI-3000C, John Morris Scientific Pty Ltd, LI-COR Biosciences), and used to compute the leaf area index (LAI). At anthesis, (GS61), the above sampling procedure was repeated. The average tiller number, ear number and AGDM per plant and per unit area were recorded. The tiller number per m^2 at GS32 and GS61 and ear number per m^2 at GS61 were determined using a plant population of 187 plants per m^2.

At maturity (GS92), plants within a 0.3 m^2 quadrat were hand harvested and processed for tiller number and ear number. The ears were oven dried at 40^0C for 48 hours, threshed and grains weighed for grain yield and thousand grain weight (TGW). The straw and grain were ground through a 0.5 mm sieve using a Thomas-Wiley Laboratory Mill (Model 4, USA, Thomas Scientific) and analysed for N concentration using a Thermo Finnigan EA 1112 Series Flash Elemental Analyser at the Central Science Laboratory, University of

Tasmania. N concentration was analysed for only the rainfed and waterlogged urea and CRF N treatments.

The N content was used to compute N uptake efficiency (NupE) (ratio of total plant N to N supply), N utilization efficiency (NutE) (ratio of grain yield to total plant N uptake) (Moll et al. 1982; Sadras & Lawson 2013), and grain protein content (GPC) (%N x 5.83), which is an indicator of grain quality (Rhee 2001). Total N uptake was calculated as (grain yield x %N in the grain + straw yield x %N in the straw)/100 (Grant et al. 1991). Nitrogen use-efficiency (NUE) (ratio of grain yield to N supply) (Fischer et al. 2014; Moll et al. 1982), N harvest index (NHI) (ratio of grain N to total plant N uptake) (Hirel et al. 2007), and harvest index (HI) (ratio of grain yield to total above-ground biomass) (Condon & Giunta 2003), were also determined. Nitrogen supply in this study refers to the amount of mineral fertiliser applied. No attempt was made to account for N mineralisation during the cropping season (Ortiz-Monasterio et al. 1997) and NUE is expressed as yield per kilogram of fertiliser-applied nitrogen (kg/kgN) only without considering the contribution of indigenous soil nitrogen (Wortmann et al. 2011). The gross margins on fertiliser use were calculated based on the prevailing wheat grain price of AU$220 /t (DPIPWE 2017). Seed and fertiliser costs were taken into account, keeping other input factors constant. The fungicide treated seed was purchased at AU$35.45 /25kg while urea and the CRF (Agrocote) were purchased at AU$30.15 /25kg and AU$55 /25kg, respectively.

Data were analysed using two-way ANOVA to determine treatment effects and interactions using GenStat 17th edition (GenStat 64-bit Release 17.1, VSN International Ltd). Treatment means were deemed significant at 5% least significant difference (LSD).

4.4 Results

During waterlogging, the depth to the water table varied from 14 to 20 cm while the rainfed treatment had its depth to the water table vary from 58 to 88 cm (Figure 4.2). There were fluctuations in the depth to the water table for the rainfed treatment, associated with changes in the daily weather patterns.

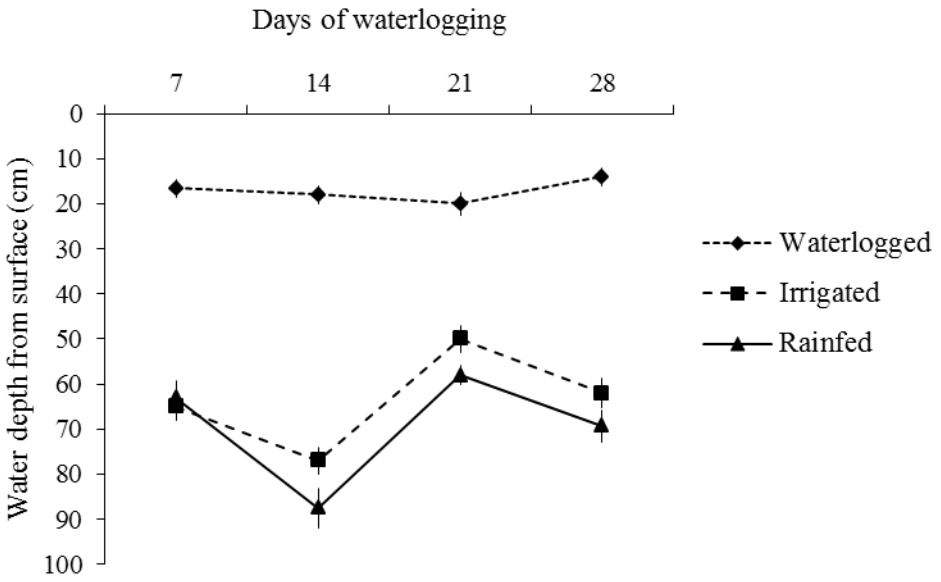

Figure 4.2: Depth of water from the surface during waterlogging. Bars indicate s.e. (n = 3).

At GS32, the interaction between irrigation regime and N fertiliser application was significant for tiller number (P = 0.013) and leaf area (P = 0.014) per plant as well as the LAI and tiller number per m^2 ($P < 0.05$). The AGDM per plant was significantly affected by irrigation regime (P = 0.001) and N fertiliser application (P = 0.012) while AGDM per unit area was significantly affected by the irrigation regime only (P = 0.001), see Table 4.2 below. Waterlogging decreased tiller number (Figure 4.3), leaf area (Figure 4.5), and AGDM per plant and per unit area (Table 4.3). For example, the average leaf area per plant for nil N treatment was 4 cm^2 under waterlogged conditions compared with 115 cm^2 and 150 cm^2 of the rainfed and irrigated regimes, respectively (Figure 4.5). The LAI (Figure 4.6) and tiller number per m^2 (Figure 4.4) were also significantly reduced by waterlogging.

The average LAI of the waterlogged plants was 0.2 compared with 4 and 3 of the rainfed and irrigated treatments, respectively. The tiller number per m^2 was also about 50% less than that of the rainfed and irrigated regimes. Nitrogen fertiliser application improved tiller number, leaf area and AGDM per plant. The CRF had more tillers in all irrigation regimes and significantly improved leaf area for the rainfed (321 cm^2/plant) and irrigated (214 cm^2/plant) regimes. It also had the greatest amount of AGDM per plant for all irrigation regimes. However, the CRF was not significantly different from single- and split-applied urea under all irrigation regimes (Table 4.3). Similarly, single-applied urea was not statistically different from split-applied urea for all parameters measured under all irrigation regimes.

Table 4. 2: Summary of the ANOVA probability values for the different growth attributes assessed during stem elongation (GS32)

Parameter	Irrigation regime	Nitrogen treatment	Irrigation regime * Nitrogen treatment
Tiller number/plant	*P* = 0.001	*P* = 0.001	*P* = 0.013
Tiller number/m^2	*P* = 0.001	*P* = 0.001	*P* = 0.013
LA/plant (cm^2)	*P* = 0.001	*P* = 0.001	*P* = 0.014
LAI	*P* = 0.001	*P* = 0.001	*P* = 0.014
AGDM/plant (g)	*P* = 0.001	*P* = 0.012	*P* = 0.278 (ns)
AGDM (g/m^2)	*P* = 0.001	*P* = 0.142 (ns)	*P* = 0.459 (ns)

Key: ns = not significant, *P* = probability, * = interaction.

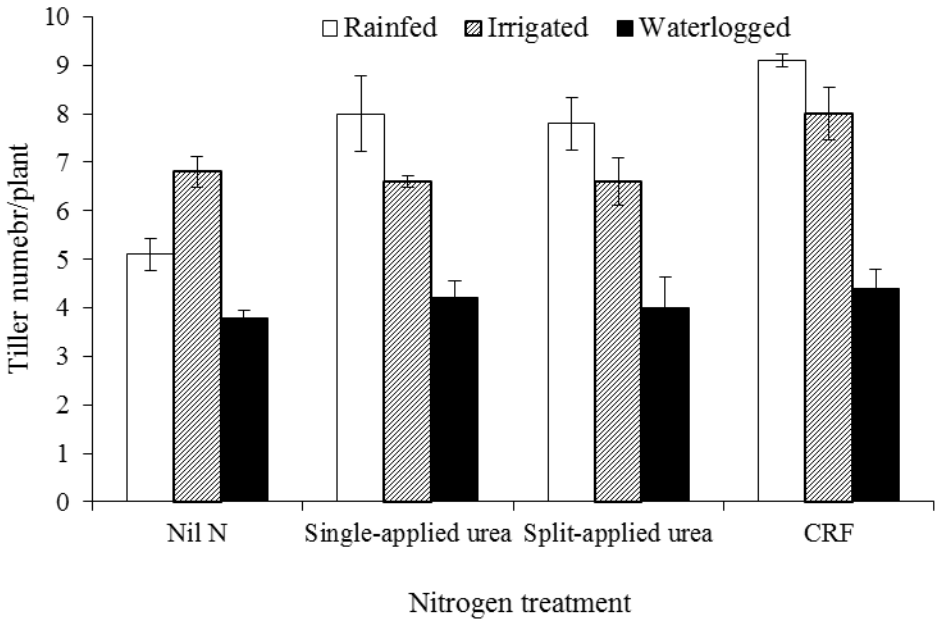

Figure 4.3: Average tiller number per plant for the different nitrogen treatments under different irrigation regimes at GS32 at Cressy Research and Demonstration Station in 2014 (P = 0.013). Bars indicate s.e. (n = 3).

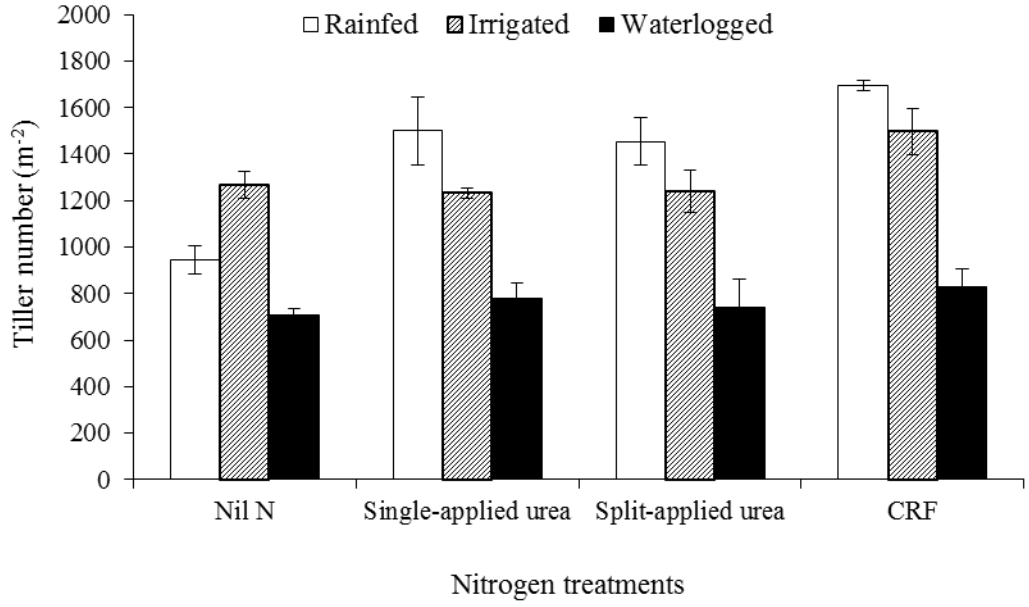

Figure 4.4: Average tiller number per m^2 for the different nitrogen treatments under different irrigation regimes at GS32 at Cressy Research and Demonstration Station in 2014 (P = 0.013). Bars indicate s.e. (n = 3).

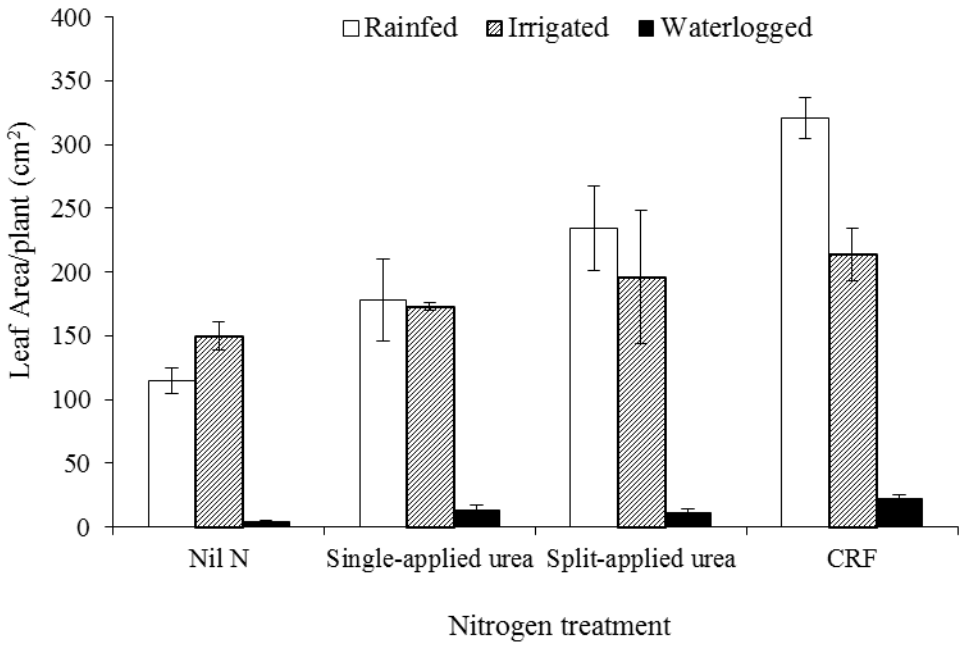

Figure 4.5: Average leaf area per plant for the different nitrogen treatments under different irrigation regimes at GS32 at Cressy Research and Demonstration Station in 2014 (P = 0.014). Bars indicate s.e. (n = 3).

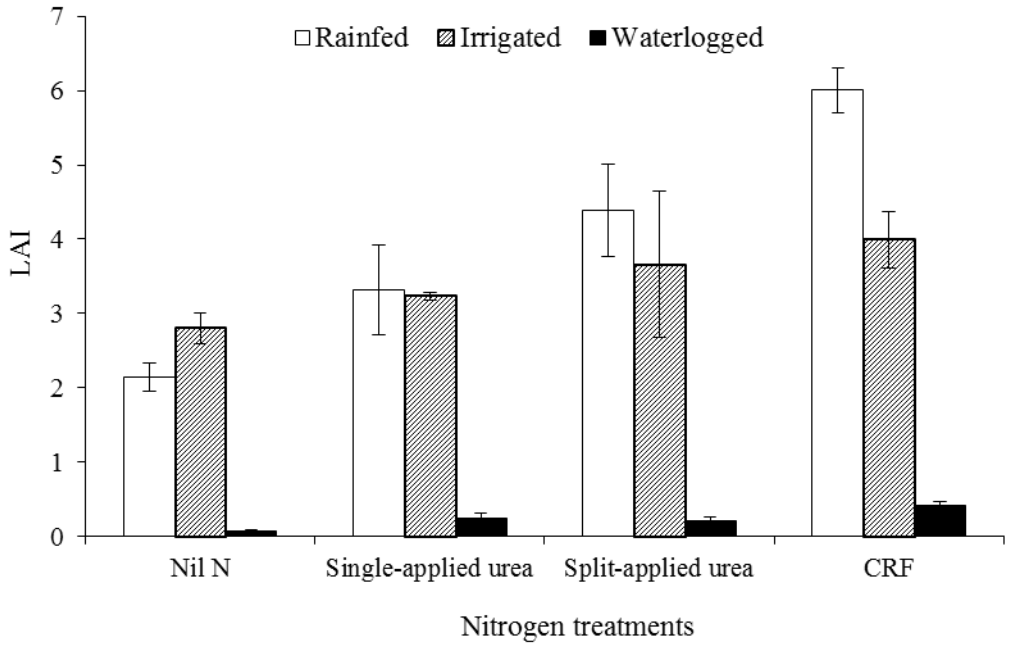

Figure 4.6: Average LAI for the different nitrogen treatments under different irrigation regimes at GS32 at Cressy Research and Demonstration Station in 2014 (P = 0.014). Bars indicate s.e. (n = 3).

Table 4. 3: Average AGDM for different irrigation regimes and nitrogen treatments at stem elongation (GS32) at Cressy Research and Demonstration Station in 2014

Source	AGDM/plant (g)	AGDM/unit area (g/m^2)
Rainfed	2.20	270
Irrigated	1.73	200
Waterlogged	0.51	72
l.s.d.	0.30	51
Nil N	1.13	139
Single-applied urea	1.51	200
Split-applied urea	1.54	186
CRF	1.74	197
l.s.d.	0.35	59

*Only main treatment effects are presented because the interaction was not significant ($P > 0.05$).

At GS61, irrigation regime ($P = 0.001$) and N fertiliser application ($P < 0.05$) had a significant effect on the tiller number, ear number and AGDM per plant and ear number and tiller number per m^2 (Table 4.5). There was no significant interaction between the irrigation regime and N fertiliser application ($P > 0.05$) for all parameters measured except for the AGDM per unit area (Table 4.4). The rainfed and irrigated regimes had a higher number of tillers, ears and AGDM per plant and ear number and tiller number per m^2 compared with the waterlogged for all respective N treatments. Nitrogen fertiliser slightly improved the number of tillers, ears and AGDM for all irrigation regimes. The CRF had the greatest number of tillers, ears and AGDM for plants under waterlogged conditions, though not statistically significant from both urea treatments. Single- and split- applied urea were not significantly different from each other under rainfed and irrigated conditions as well for all parameters measured. Figure 4.7 shows the AGDM per unit area for the different N treatments under rainfed, irrigated and waterlogged conditions.

Table 4. 4: Summary of the ANOVA probability values for the different growth attributes assessed at anthesis (GS61)

Parameter	Irrigation regime	Nitrogen treatment	Irrigation regime * Nitrogen treatment
Tiller number/plant	*P* = 0.001	*P* = 0.003	*P* = 0.503 (ns)
Tiller number/m^2	*P* = 0.001	*P* = 0.003	*P* = 0.503 (ns)
Ear number/plant	*P* = 0.001	*P* = 0.005	*P* = 0.391 (ns)
Ear number/m^2	*P* = 0.001	*P* = 0.005	*P* = 0.391 (ns)
AGDM/plant (g)	*P* = 0.001	*P* = 0.014	*P* = 0.812 (ns)
AGDM (g/m^2)	*P* = 0.001	*P* = 0.175 (ns)	*P* = 0.031

Key: ns = not significant, *P* = probability, * = interaction.

Table 4. 5: Growth attributes for different irrigation regimes and nitrogen treatments at anthesis (GS61) at Cressy Research and Demonstration Station in 2014

Source	Tiller number /plant	Tiller number /m^2	Ear number /plant	Ear number /m^2	AGDM /plant (g)
Rainfed	4.9	922	4.4	822	15.6
Irrigated	5.1	947	4.9	918	17.2
Waterlogged	2.9	534	2.6	486	5.8
l.s.d.	0.5	89	0.4	75	1.7
Nil N	3.6	672	3.4	639	10.8
Single-applied urea	4.4	828	4.0	753	13.6
Split-applied urea	4.6	861	4.2	779	13.4
CRF	4.5	843	4.3	796	13.8
l.s.d.	0.6	103	0.5	87	1.9

*Only main treatment effects are presented because the interaction was not significant (*P* > 0.05).

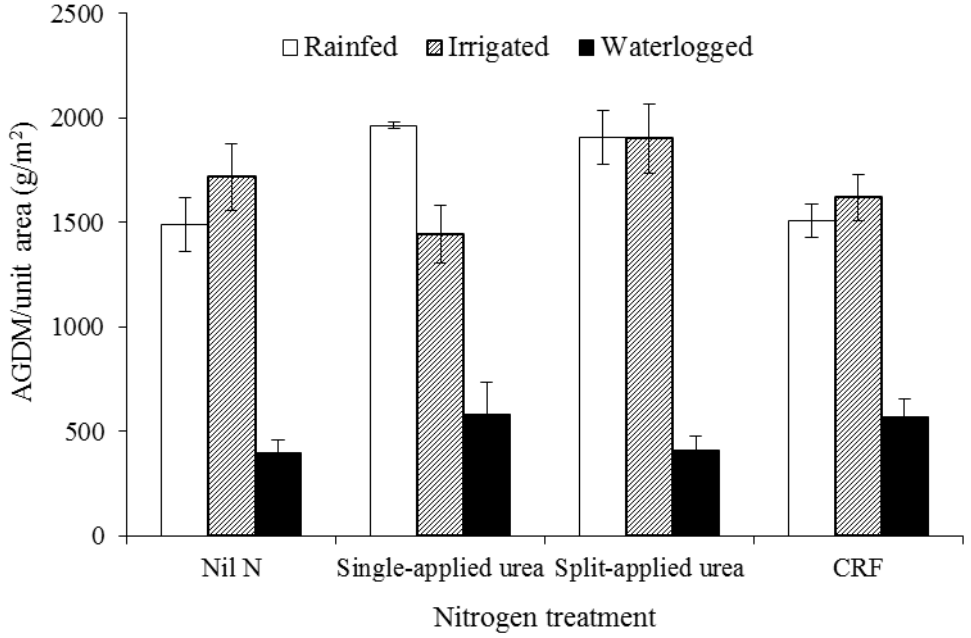

Figure 4.7: AGDM per unit area for the different nitrogen treatments under different irrigation regimes at anthesis (GS61) at Cressy Research and Demonstration Station in 2014 (P = 0.03). Bars indicate s.e. (n = 3).

At maturity (GS92), yield attributes including: tiller number, ear number and grain yield per unit area were significantly affected by the irrigation regime (P = 0.001) and N fertiliser application ($P < 0.05$) (Table 4.6). The rainfed regime had the highest number of tillers compared with respective N treatments of the irrigated and waterlogged. Nitrogen fertiliser application improved tiller number, with single-applied urea of the rainfed having the highest number of tillers (692 tillers /m^2) (Table 4.7). The CRF had the highest number of tillers for the irrigated (639 tillers /m^2) and waterlogged (526 tillers /m^2). Similarly, ear number was improved by N fertiliser application; the CRF still had the highest number of ears for the irrigated (603 ears /m^2) and waterlogged (477 ears /m^2) while single-applied urea (686 ears /m^2) had the greatest number of ears for the rainfed (Table 4.7). Grain yield increased in response to N fertiliser application under all irrigation regimes. The waterlogged plants had the lowest grain yield compared with their respective N treatments for the rainfed and irrigated. The CRF had the highest grain yield for all irrigation regimes

with 9.2 t/ha, 9.4 t/ha and 6.8 t/ha for the rainfed, irrigated and waterlogged, respectively (Table 4.7). The urea treatments had an average of 8 t/ha for both the rainfed and irrigated, and 5.3 t/ha for the waterlogged. The CRF increased grain yield by an average of 1.5 t/ha compared with both urea treatments under waterlogged conditions.

There was a significant interaction between irrigation regime and N application for the harvest index (HI) ($P = 0.04$) (Figure 4.8) and thousand grain weight (TGW) ($P = 0.03$) (Figure 4.9). The waterlogged plants had the highest HI for all N treatments compared with their respective N treatments for the rainfed and irrigated. Single-applied urea had the highest HI, 0.54 though no significant statistical differences were noted between N treatments of the different irrigation regimes. Both urea and CRF treatments had higher harvest indices than nil N treatments of the rainfed and irrigated. Interestingly, nil N of the waterlogged had a HI of 0.52, higher than the split-applied urea and CRF and other N treatments of the rainfed and irrigated plants. The TGW was relatively similar for most N treatments under rainfed and irrigated conditions. The nil N treatment of the waterlogged had the lowest TGW of 36 g compared with other N treatments, which had an average of 42 g.

Table 4. 6: Yield attributes and NUE for different irrigation regimes and nitrogen treatments at maturity (GS92) at Cressy Research and Demonstration Station in 2014

Source	Tiller number/m^2	Ear number/m^2	Grain yield (t/ha)	NUE (kg/kgN)
Rainfed	650	609	7.2	91.7
Irrigated	584	566	8.0	94.7
Waterlogged	395	372	5.2	63.9
l.s.d.	68	63	1.0	13.5
Nil N	467	426	4.6	-
Single-applied urea	536	524	7.2	79.8
Split-applied urea	563	541	6.9	76.7
CRF	607	571	8.5	93.9
l.s.d.	79	73	1.2	13.5

*Only main treatment effects are presented because the interaction was not significant ($P > 0.05$).

Table 4. 7: Interactions between irrigation regime and nitrogen fertilser application for yield attributes and NUE at maturity (GS92) at Cressy Research and Demonstration Station in 2014

Irrigation regime	Nitrogen treatment	Tiller number/m^2	Ear number/m^2	Grain yield (t/ha)	Harvest Index (ratio)	NUE (kg/kgN)
Rainfed						
	Nil N	604cd	492abcd	3.8a	0.30a	-
	Single-applied urea	692d	686d	7.7bc	0.41ab	85ab
	Split-applied urea	647cd	627d	7.8bc	0.42ab	87ab
	CRF	657cd	632d	9.2c	0.48b	103b
Irrigated						
	Nil N	516abcd	513bcd	6.5abc	0.42ab	-
	Single-applied urea	563bcd	551bcd	8.3bc	0.45ab	93ab
	Split-applied urea	617cd	594cd	7.9bc	0.42ab	88ab
	CRF	639cd	603cd	9.4c	0.49b	104b
Waterlogged						
	Nil N	280a	272a	3.6a	0.52b	-
	Single-applied urea	351ab	336ab	5.6ab	0.54b	62a
	Split-applied urea	424abc	403abc	5.0ab	0.50b	55a
	CRF	526bcd	477abcd	6.8abc	0.48b	75ab
	l.s.d.	137	126	2.0	0.1	23.4

*Treatments followed by the same letter are not significantly different ($P > 0.05$).

Nitrogen-use efficiency was significantly affected by irrigation regime ($P = 0.001$) and N fertiliser application ($P = 0.036$). The waterlogged plants had the lowest NUE for all respective N treatments under the rainfed and irrigated regimes. The CRF had the highest NUE for all irrigation regimes; over all, the irrigated regime had the highest NUE for both urea and CRF treatments (Table 4.6). Under waterlogged conditions, CRF improved NUE by 17% and 27% more than single- and split- applied urea, respectively.

There were no significant variations between N treatments for the different NUE components assessed and GPC (Table 4.8). The irrigation regime had a significant effect on NupE, NutE, NHI and GPC ($P < 0.05$). The rainfed had higher NupE than the waterlogged for all N treatments while the waterlogged had higher NutE and NHI for all N treatments compared with the rainfed. The GPC was relatively similar for both the rainfed and waterlogged. Single-applied urea had the lowest GPC with 12% and 9% for the rainfed and waterlogged, respectively (Table 4.9).

The gross margins generated on fertiliser use were significantly affected by the irrigation regime and N fertiliser application ($P < 0.05$) (Table 4.8). The irrigated regime generated the highest margin with 14% and 46% more than the margins generated under rainfed and waterlogged conditions, respectively. While the gross margins generated by single-applied urea, split-applied urea and the CRF were not significantly different, nil N application resulted in about 27% less of the gross margins obtained with both urea treatments and the CRF.

Table 4. 8: NUE components, GPC and gross margins on fertiliser use for different irrigation regimes and nitrogen treatments at maturity (GS92) at Cressy research and Demonstration Station in 2014

Source	NupE (ratio)	NutE (ratio)	NHI (ratio)	GPC (%)	Gross margin (AU$/ha)
Rainfed	2.57	36	0.75	12.6	1,146
Irrigated	-	-	-	-	1,337
Waterlogged	1.33	50	0.80	9.6	723
l.s.d.	0.56	9	0.05	2.4	224
Nil N	-	-	-	-	837
Single-applied urea	1.73	47	0.79	10.4	1,163
Split-applied urea	1.78	42	0.78	11.4	1,103
CRF	2.34	40	0.76	11.5	1,171
l.s.d.	0.70	11	0.06	2.9	258

*Only main treatment effects are presented because the interaction was not significant ($P > 0.05$).

Table 4. 9: Interactions ($P > 0.05$) between irrigation regime and nitrogen fertiliser application for NUE components and GPC at maturity (GS92) at Cressy Research and Demonstration Station in 2014

Irrigation regime	Nitrogen treatment	NupE (ratio)	NutE (ratio)	NHI (ratio)	GPC (%)
Rainfed					
	Nil N	-	-	-	-
	Single-applied urea	2.34ab	38.2a	0.76a	12a
	Split-applied urea	2.42ab	34.3a	0.75a	13a
	CRF	2.94b	36.0a	0.74a	13a
Waterlogged					
	Nil N	-	-	-	-
	Single-applied urea	1.11a	56.6a	0.83a	9a
	Split-applied urea	1.13a	48.8a	0.80a	10a
	CRF	1.74ab	43.5a	0.78a	11a
	l.s.d.	1.0	16	0.1	4.1

*****Treatments followed by the same letter are not significantly different ($P > 0.05$).

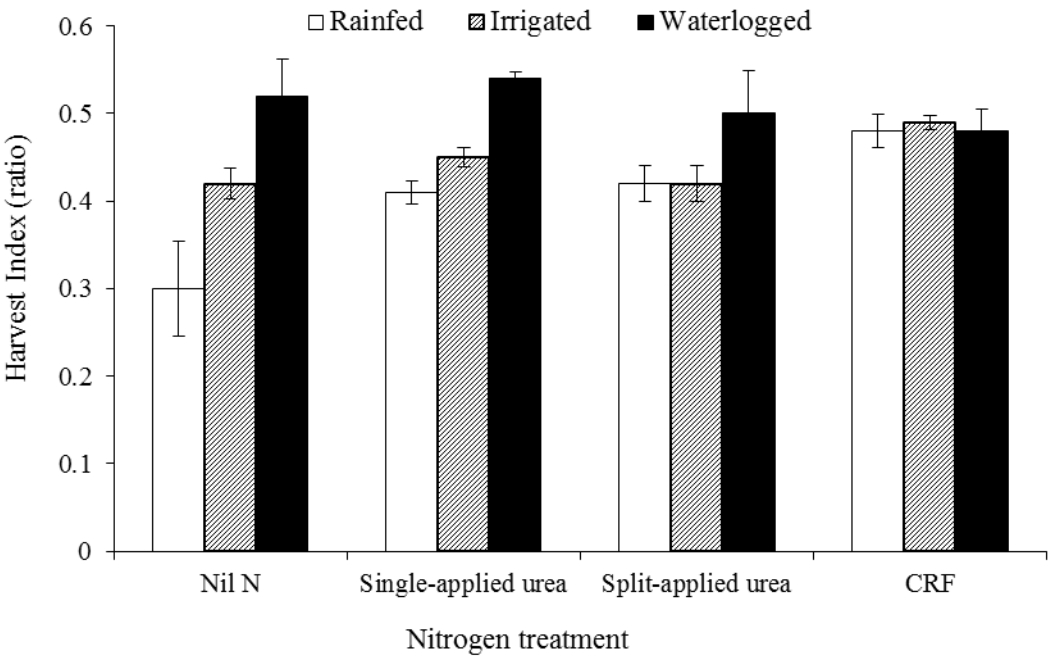

Figure 4.8: Harvest index (HI) for the different nitrogen treatments under different irrigation regimes at maturity (GS92) at Cressy Research and Demonstration Station in 2014 (P = 0.04). Bars indicate s.e. (n = 3).

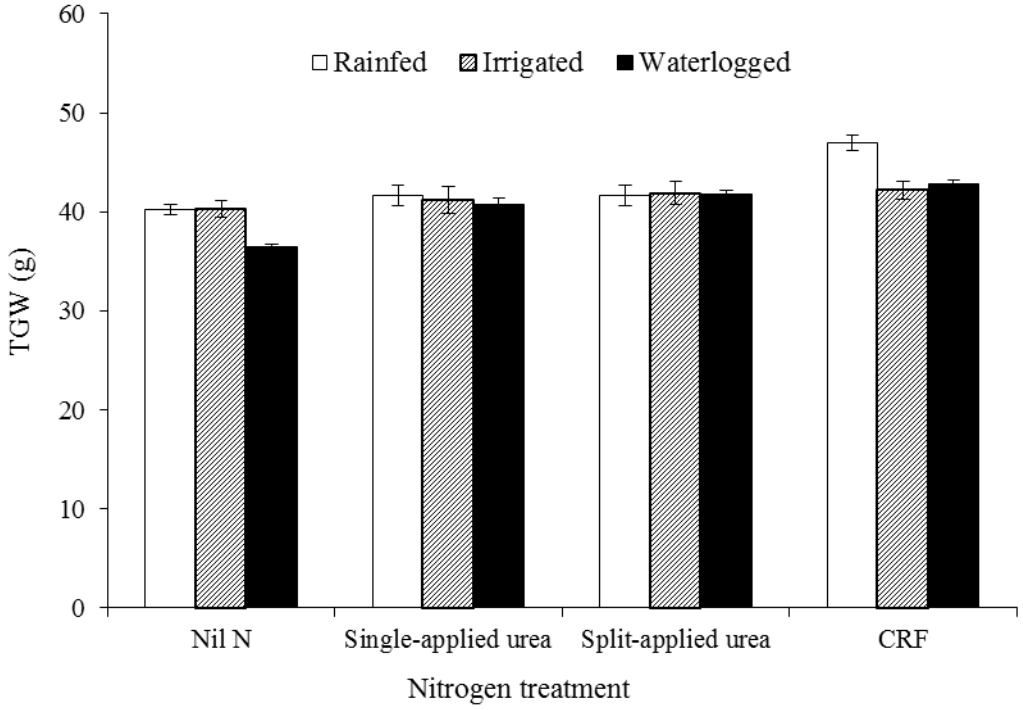

Figure 4.9: Thousand grain weight (TGW) for the different nitrogen treatments under different irrigation regimes at maturity (GS92) at Cressy Research and Demonstration Station in 2014 (P = 0.03). Bars indicate s.e. (n = 3).

Table 4. 10: Summary of the ANOVA probability values for the different yield attributes, NUE components, GPC and gross margins assessed at maturity (GS92)

Parameter	Irrigation regime	Nitrogen treatment	Irrigation regime * Nitrogen treatment
Tiller number/m^2	*P* = 0.001	*P* = 0.01	*P* = 0.365 (ns)
Ear number/m^2	*P* = 0.001	*P* = 0.003	*P* = 0.343 (ns)
Grain yield (t/ha)	*P* = 0.001	*P* = 0.001	*P* = 0.44 (ns)
TGW (g)	*P* = 0.007	*P* = 0.001	*P* = 0.026
Harvest Index (HI)	*P* = 0.001	*P* = 0.053	*P* = 0.039
NUE (kg/kgN)	*P* = 0.001	*P* = 0.036	*P* = 0.984 (ns)
NupE (ratio)	*P* = 0.001	*P* = 0.150 (ns)	*P* = 0.99 (ns)
NutE (ratio)	*P* = 0.008	*P* = 0.329 (ns)	*P* = 0.568 (ns)
NHI (ratio)	*P* = 0.029	*P* = 0.433 (ns)	*P* = 0.734 (ns)
GPC (%)	*P* = 0.018	*P* = 0.635 (ns)	*P* = 0.874 (ns)
Gross margin (AU$/ha)	*P* = 0.001	*P* = 0.044	*P* = 0.44 (ns)

Key: ns = not significant, *P* = probability, * = interaction.

4.5 Discussion

Findings from this study showed that waterlogging adversely affects the growth and development of wheat. This is consistent with results from previous studies by Zhang et al. (2006); Zhang et al. (2004); Zhou (2010). In duplex soils, this is attributed to the formation of perched water tables on the subsoil clay causing excess water within the crop root zone (Bakker et al. 2005); depleting the soil O_2 (Condon & Giunta 2003) and substantially diluting nutrient concentrations (Pang et al. 2007). This decreases the availability of essential plant nutrients, leaving plants with marked nutritional deficiency symptoms (Huang et al. 1994b; Trought & Drew 1980). Such symptoms include chlorosis, which accelerates premature leaf senescence thereby decreasing the plant canopy, photosynthetic capacity and resultant biomass. Waterlogging also decreases root biomass through restricted root growth and death of seminal root tips, which impairs the ability of plants to forage for the already limited resources (Pang et al. 2007). Moreover, under stressful

conditions, plants channel their resources (photoassimilates) to key plant organs such as the grains and maintenance of vital physiological processes for survival. This improves their productivity as can be observed with the higher harvest indices, NHI and N utilisation efficiency obtained under waterlogged conditions in this study.

For this particular experiment (2014/2015), the study findings showed that an average yield of 8 t/ha can be obtained under rainfed conditions with conventional urea. Similar average wheat yields were reported by Acuña et al. (2011) for high yielding varieties like Revenue in the high rainfall environment in Tasmania. Such high yields under rainfed conditions can be attributed to the cool-temperate climate with over 550 mm of rainfall annually (Mathers et al. 2007), that is sometimes sufficient for wheat growth precluding the need for irrigation. Also the longer growth season in the HRZ provides time for more tillers to establish and develop a full canopy to maximize incoming solar radiation (Riffkin & Sylvester-Bradley 2008) and productivity. The rainfed treatment also had the greatest number of tillers and ears per unit area, which possibly increased the number of fertile heads and thus grain yield. In the HRZ, an average of 500 ears per m^2 should be targeted to give the crop the chance to achieve its potential yield since wheat yield is significantly correlated with plant population density or tiller number and ear number (Zhang et al. 2007). Besides, Revenue as a variety is known to be high yielding (Acuña et al. 2011). Nonetheless, irrigation is vital to ensure adequate soil moisture availability for plant growth and maximize wheat yields in the HRZ of southern Australia, which are predicted at ~12 t/ha (Acuña et al. 2011).

Consistent with studies by Fois et al. (2009); Mohammed et al. (2013); Wang et al. (2012), the results showed that wheat cv. Revenue was responsive to N fertiliser application. Nitrogen is a vital macronutrient for plant growth (Mengel & Kirkby 2001), with a

significant proportion allocated to the chloroplasts for synthesis of components for the photosynthetic apparatus (Hirel et al. 2007). Nitrogen uptake increases leaf chlorophyll content and photosynthetic capacity (Wang et al. 2012), which propels vegetative growth (tillering and canopy size and duration); resultant grain yield and biomass (Cai et al. 2012; Fois et al. 2009). Under waterlogged field conditions, the results showed that N fertiliser application improved wheat growth and yield. Robertson et al. (2009), also reported similar findings where N fertiliser applied after waterlogging increased grain yield by ~20%. Similarly, increased rates of top-dressed urea were reported to significantly increase wheat grain yield on flooded sodic soils in India (Swarup & Sharma 1993). The role of N availability particularly in the form of NO_3^- ions in alleviating the effects of waterlogging was emphasised by Drew (1991). Nitrate ions can replace molecular O_2 as a terminal acceptor thereby sustaining respiration and cell survival during anoxia through dissimilatory NO_3^- reduction. On the contrary, the efficiency of N fertiliser is usually reduced by waterlogging when significant amounts are lost through leaching and denitrification (Noellsch et al. 2009). Appropriate timing of N fertiliser application is important; single-applied urea had a significant amount of N available at sowing, which accelerated plant vegetative growth and offset the adverse effects of waterlogging. Top-dressing urea on the otherhand, boosts plant growth after waterlogging with the production of secondary tillers that produce more late ears (Robertson et al. 2009). It also increases canopy duration and accelerates the production of photoassimilates translocated to the grain compared with the straw thus increasing the HI. Nonetheless, top-dressing urea after waterlogging can be difficult under field conditions as the soil moisture content is often too high for efficient mechanization. Interestingly, CRFs can be applied in a single application at sowing, saving significant labour costs, time and energy (Lubkowski & Grzmil 2007; Mathers et al. 2007). In addition, a 20-30% reduction in the recommended

conventional fertiliser application rate has been suggested when applying SR/CR fertiliser without incurring any yield penalty (Trenkel 2010). This together with the availability of CRFs with different nutrient release periods allows farmers to tailor specific CRF products to the appropriate crop and farming conditions or predicted weather conditions depending on the season. Given Tasmania's temperate climate, CRF products with three or four and six month release periods would be appropriate for short- and long-season crops grown over the spring and winter seasons, respectively.

The study findings also showed that CRF improved wheat grain yields and NUE under different irrigation regimes. This is supported by previous studies by Wenyu et al. (2005), which revealed that controlled-release urea (CRU) can increase wheat grain yield, protein content and markedly reduce NO_3^- leaching and Shoji et al. (2001), who obtained a total N recovery of 66% in flood irrigated barley grown on a clay soil in north eastern Colorado, USA. CRFs release N over an extended period of time during crop growth (Lubkowski & Grzmil 2007; Trenkel 2010) and maximize NUE by synchronizing N release with crop demand (Chen et al. 2008; Trenkel 2010). This was reflected in the gross margins on fertiliser use where the CRF generated more returns than single- and split-applied urea. However, the increase was not significant to warrant investment taking into account the higher cost associated with CRFs.

Apart from N source, NUE is also significantly influenced by water availability (Asseng et al. 2001). Irrigation plays a crucial role in improving NUE (Fischer et al. 1993), particularly under Mediterranean and dry farming environments. In the temperate regions like Tasmania, the moderate to high annual rainfall sometimes precludes the need for irrigation especially for cereals, which are of low value relative to vegetables and horticultural crops like poppies. This could justify the high NUE of 92 kg/kgN observed

under rainfed conditions, which was not significantly different from that of the irrigated regime, 95 kg/kgN. Waterlogging associated with N loss particularly through leaching and denitrification decreased NUE to almost 32% of the rainfed and irrigated treatments. NUE is also influenced by N uptake and utilisation processes and N supply (Moll et al. 1982). At low N supply or availability, NUE is influenced by the utilisation of accumulated N while at high N supply; differences in NUE are largely due to variation in N uptake efficiency. The efficiency of N uptake and utilization requires effective operation of processes associated with absorption, translocation, assimilation and redistribution of N (Moll et al. 1982).

While the use of conventional urea in mitigating the adverse effects of waterlogging has been explored particularly under field conditions (Robertson et al. 2009; Swarup & Sharma 1993; Wang et al. 2012), the potential of CRFs in broadacre cropping and as an option in improving plant growth and development as well as NUE under waterlogged conditions has not been exploited. Findings in this study showed that CRFs can improve wheat growth and NUE under waterlogged conditions. It was also observed that CRFs may not be significantly different from conventional urea. Much as the CRF improved wheat growth and yield attributes, NUE, NUE components and GPC, this improvement was not statistically significant compared with conventional urea, which did not support the hypothesis that under similar irrigation management, the CRF will significantly increase wheat growth attributes, yield components, NUE, NUE components and GPC compared with the single- and split-applied urea treatments. Appropriate timing of N fertiliser application was also shown to be important; while top-dressing urea after waterlogging can help plants compensate for the adverse effects of waterlogging with the production of secondary tillers, which often develop late ears, application single application of urea too at sowing can enhance vegetative growth and help plants withstand waterlogging stress.

4.6 Conclusion

Although waterlogging is still a major abiotic constraint to wheat production, N fertiliser application could improve wheat yield. The timing of N application and source are important. Applying full amount of the required fertiliser at sowing helps plants to withstand the adverse effects of transient and intermittent waterlogging through enhanced vegetative growth. Using CRFs may improve wheat growth and NUE under rainfed and waterlogged conditions though there might be no significant yield advantage over conventional urea to warrant investment. Nonetheless, there is need to evaluate different CRF products available for their potential in broadacre cropping and understand the processes involved in improving NUE and how they can be enhanced to maximize their productivity. This will also further underscore the potential of CRFs in reducing N_2O emissions (Akiyama et al. 2010; Burney et al. 2010; Jiang et al. 2010; Smith et al. 2007), particularly under waterlogged conditions and the need to reduce prices of these fertilisers. The next chapter maps the uptake of NH_4^+, NO_3^- and H^+ ions along the root axis of selected wheat and barley varieties under hypoxia in order to shed light on the ionic mechanisms underlying the effect of waterlogging on plant nutrient acquisition across the plasma membrane of epidermal root cells in physiologically different root zones.

Chapter Five: Mapping ammonium (NH_4^+) and nitrate (NO_3^-) uptake under hypoxia in cereals

5.1 Abstract

Ammonium (NH_4^+) and nitrate (NO_3^-) are the predominant sources of nitrogen (N) available for plant nutrition. This study characterised the uptake of NH_4^+, NO_3^- and H^+ along the root axis of selected wheat and barley varieties under hypoxia. Steady-state net fluxes of NH_4^+, NO_3^- and H^+ ions were measured at different positions along the root axis of wheat cv. Revenue and barley cv. Naso Nijo using non-invasive microelectrode ion flux estimation (MIFE) technique. The results showed that the uptake and efflux of NH_4^+ and NO_3^- ions in wheat and barley varied significantly (P = 0.001) between different positions along the root axis at 20 µM and 1 mM NH_4NO_3 during normoxia and hypoxia. In wheat, positions above 0.5 mm from the root tip were more sensitive to hypoxia at 20 µM with NH_4^+ efflux occurring across all positions. However, at 1 mM, the elongation and mature zones were less sensitive to hypoxia with NH_4^+ uptake occurring across all positions. For NO_3^-, positions between 0.5-20 mm from the root tip were less sensitive to hypoxia at 20 µM. At 1 mM, the elongation and mature zones were less sensitive to hypoxia with NO_3^- uptake occurring across all positions on the root axis. The highest uptake at occurred at 2.5 mm from the root tip with an average of 270 µmol m^{-2} s^{-1}. For barley, NH_4^+ uptake at 20 µM was less sensitive to hypoxia in the elongation zone with an average uptake of 175 µmol m^{-2} s^{-1} at 0.5 and 1.5 mm from the root tip. The mature zone was more sensitive to hypoxia with NH_4^+ efflux occurring across all positions at an average of -34 µmol m^{-2} s^{-1}. However, at 1 mM, NH_4^+ uptake occurred across all positions along the root axis. At 20 µM, the NO_3^- ion experienced minimal efflux across different positions along the root axis with the exception of positions at 0.5 and 50 mm from the root tip. At 1 mM, the

elongation zone registered the highest uptake of NO_3^- ion at an average of 16 μmol m^{-2} s^{-1} compared with 9 μmol m^{-2} s^{-1} of the mature zone. Hypoxia increased the uptake of NH_4^+ and NO_3^- for both wheat and barley and there was a preference for NH_4^+ over NO_3^-. Genotypic differences between wheat and barley were evident, with wheat having the highest uptake of both NH_4^+ and NO_3^- for the control and hypoxia at 20 μM and 1 mM.

Key words: Hypoxia; Ammonium; Nitrate; Uptake; *Triticum aestivum L.*; *Hordeum vulgare* L.

5.2 Introduction

In most soils, ammonium (NH_4^+) and nitrate (NO_3^-) are the predominant sources of nitrogen (N) available for plant nutrition (von Wirén et al. 2000; Yan et al. 2011). The charge of the ion strongly influences whether transport will be thermo-dynamically favourable or require the expenditure of additional metabolic energy (Haynes 1990; Henriksen et al. 1992). It also determines the methods needed to maintain electroneutrality at the root-soil interface (Haynes 1990; Henriksen et al. 1992). The assimilation of each ion affects the cytoplasmic pH differently; the incorporation of NH_4^+ generates H^+ whereas the reduction of NO_3^- results in a net alkalinisation of the cytosol (Henriksen et al. 1992). When both NH_4^+ and NO_3^- are present in a nutrient solution, NH_4^+ is taken up preferentially to NO_3^- (Alexandre et al. 2011; Botella et al. 1994) for the synthesis of amino acids and proteins (Schrader et al. 1972). Nonetheless, higher rates of total N uptake often occur when both NH_4^+ and NO_3^- are present in a nutrient solution compared with a single source, which leads to better plant growth and development (Botella et al. 1994).

Genotypic differences in N uptake exist, with some showing preference for one form of N over the other (Glass 1988). The plasticity of different species towards N source indicates that most species show higher plasticity for NH_4^+ especially under low nutrient availability

(Tylova-Munzarova et al. 2005). This sensitivity to the different forms of N depends on N availability within the rhizosphere and soil pH (Tylova-Munzarova et al. 2005). Under hypoxic conditions, plants show preference to NH_4^+ over NO_3^- (Garnett et al. 2001; Kronzucker et al. 2000; Yan et al. 2011). Ammonium can be absorbed and utilized directly by many plant species of higher plants (Arnon 1937; von Wirén et al. 2000). It also easily adsorbs to soil colloids while the NO_3^- is readily leached (Buwalda & Greenway 1989). Furthermore, anaerobic conditions cause substantial reduction in net NO_3^- uptake due to denitrification and leaching and restriction of nitrification (Buwalda et al. 1988; Buwalda & Greenway 1989). The reduction of NO_3^- to NO_2 by nitrate reductase (NR) and subsequently to NH_4^+ by nitrite reductase (NiR) increases the availability of NH_4^+ during hypoxia (Bailey-Serres & Voesenek 2008; Daniel-Vedele et al. 1998).

Nonetheless, hypoxia decreases the status of N in plants (Buwalda & Greenway 1989); O_2 deprivation has an immediate and substantial effect on root ion flux patterns and this effect differs between hypoxia tolerant and intolerant varieties. Most electrophysiological studies have focused on the physiological and molecular mechanisms associated with the uptake of NH_4^+ under hypoxia particularly in rice (*Oryza sativa*) and a model plant *Arabidopsis thaliana* (Crawford & Glass 1998; von Wirén et al. 2000). Little attention has been given to the uptake of $NH_4^+$and NO_3^- along the root axis in other cereals such as wheat (*Triticum aestivum* L.) or barley (*Hordeum vulgare* L.) under hypoxia. Yet such knowledge will allow us understand the mechanisms by which hypoxia affects plant N nutrition and offer breeders the recipes on how to fix the issue. Understanding N uptake under hypoxia is vital to designing and developing management and mitigation strategies to improve N uptake efficiency and resultant NUE under waterlogged conditions. Such strategies include breeding waterlogging tolerant wheat and barley cultivars with desirable root morphological characteristics and architecture and growth habits to maximise N uptake

and crop productivity. Mapping the uptake of NH_4^+ and NO_3^- ions will give insight into the processes and mechanisms influencing N uptake under hypoxia since N uptake varies between mature and growing (meristematic and elongation) root regions (Colmer & Bloom 1998; Henriksen et al. 1990). This study characterised the uptake of NH_4^+, NO_3^- and H^+ along the root axis of selected wheat and barley varieties under hypoxia basing on the premises that the uptake of NH_4^+, NO_3^- and H^+ will vary significantly along the root axis and between genotypes, and the uptake of NH_4^+ will be significantly higher than NO_3^-.

5.3 Materials and methods

5.3.1 Plant material and growth conditions

Wheat (cv. Revenue) and barley (cv. Naso Nijo) varieties with contrasting waterlogging tolerance abilities were used. Revenue is waterlogging tolerant while Naso Nijo is waterlogging sensitive. The seeds were surface sterilized with 5% commercial bleach (NaClO 42 gL^{-1}; Pental Products, Shepparton, VIC, Australia) for 15 minutes and thoroughly rinsed with tap water for 30 minutes. The seeds were grown in paper rolls placed in a small plastic cup with distilled water and left in darkness for three to four days at room temperature (25 ± 1^0C). The seedlings were used for experimental purposes when the roots are about 70 mm long.

5.3.2 Experimental protocol for ion flux measurements under normoxia (control)

Primary roots of ~70 mm were used for electrophysiological measurements. Seven positions were assessed along the root axis. Three positions were measured within the elongation zone and four positions were selected from the mature zone. Sections of ~3 mm and ~20 - 50 mm from the root tip were used to denote the elongation (Zeng et al. 2014) and mature zones (Pang et al. 2006), respectively. In the elongation zone, the three

positions were demarcated at 0.5, 1.5 and 2.5 mm from the root tip while in the mature zone; the four positions were demarcated at 20, 30, 40 and 50 mm from the root tip. Excised primary roots were immobilised horizontally with elastic cross-bars in 6 ml Perspex measuring chambers containing two different concentrations (treatments) of 20 µM and 1 mM NH_4NO_3 for two hours prior to taking measurements. The two concentrations were chosen because both NH_4^+ and NO_3^- exhibit a high-affinity transport system (HATS) at 20 µM and 1 mM (Glass 2003). The HATS facilitates NH_4^+ and NO_3^- uptake at low concentrations in the soil (Feng et al. 2011; Forde 2000). Additionally, the uptake of NH_4^+ and NO_3^- follows Michaelis-Menten kinetics (K_m) (Botella et al. 1994), characterised by a biphasic pattern at low concentrations and a linear non-saturating uptake at higher concentrations (Glass 2003; von Wirén et al. 2000). Five individual roots of different plants were used for each variety and concentration. The roots were excised in order to eliminate the possibility of internal oxygen transport (Pang et al. 2006).

Microelectrodes for the different ions, NH_4^+, NO_3^- and H^+ were prepared using ion-specific back filling solutions and Liquid Ion Exchangers (LIXs). The back filling solution for both NH_4^+ and NO_3^- was 500 mM NH_4NO_3 and 15 mM NaCl and 40 mM KH_2PO_4 for H^+. The prepared electrodes were placed in a basic salt medium solution (BSM), 0.5 mM KCl and 0.1 mM $CaCl_2$ for conditioning. The NH_4^+ and NO_3^- electrodes were calibrated against low and high concentrations at: 50, 100 and 200 µM NH_4NO_3 for the low concentration and 500, 1000 and 1500 µM NH_4NO_3 for the high concentration. The H^+ electrode was calibrated against pH of 5.43, 6.60 and 7.86. The specific LIX used were: H^+; 95291-0.1ML Hydrogen Ionophore 1 - Cocktail A Selectophore™ and NH_4^+; 99978-0.1ML-F Ammonium Ionophore 1 - Cocktail A Selectophore™. The NO_3^- cocktail was provided by Dr Cai Shengguan and due to confidentiality purposes, details of the ionophore cannot be divulged as it is being patented.

Steady-state net fluxes of NH_4^+, NO_3^- and H^+ ions were measured non-invasively using the microelectrode ion flux estimation (MIFE) technique for two minutes at each position. The theory of MIFE measurements and details of ion-selective microelectrode fabrication and calibration are detailed in Shabala et al. (2006). The microelectrode tips were positioned in one plane, with ~2 μm between them and 40 μm above the root surface. During the measurements, microelectrodes were moved in a slow (10s cycle) square-wave manner by a computer-driven micromanipulator between two positions close to (40 μm) and away from (120 μm) the root surface. The potential difference between two positions was recorded by the MIFE CHART software and converted to electrochemical potential difference using the calibrated Nernst slope of the electrodes (Shabala et al. 2006). Net ion fluxes were calculated from the electrochemical potential difference using cylindrical diffusion geometry by the MIFEFLUX program (University of Tasmania, Hobart, Australia). The H^+ flux was measured because thermodynamic considerations indicate that NO_3^- uptake requires an active transport system, which is proton-coupled and dependent on the H^+ pumping activity of the plasma membrane H^+-ATPase (Forde 2000).

5.3.3 Ion flux measurements under hypoxia

For measurements under hypoxia, excised primary roots were immobilised horizontally with elastic cross-bars in 6 ml Perspex measuring chambers. The chambers were submerged in containers containing a hypoxic agar solution for 48 hours. The stagnant solution was prepared by adding agar (Oxoid, Hampshire, United Kingdom) to the NH_4NO_3 solution in a ratio of 0.2% (w/v) for concentrations at 20 µM and 1 mM. The mixture was boiled and allowed to cool down over night at room temperature with a stirrer to prevent lump formation. The cool agar solution was bubbled with high purity N_2 gas (BOC Gases, Hobart, Australia) for three hours prior to being used in the experiment

(Zeng et al. 2014). The agar-treated chambers with roots were flushed with N_2 gas for one hour prior to be being kept for 48 hours. After 48 hours, the chambers were carefully removed from the hypoxic agar solution for steady-state MIFE measurements. Net ion fluxes were calculated as described above.

5.3.4 Data analysis

Data for the different wheat and barley varieties used were analysed using two-way analysis of variance (GenStat 17th edition) to determine the main effects and interactions between O_2 availability (control and hypoxia) and position along the root axis for NH_4^+, NO_3^- and H^+ ions. Differences in ion uptake for the different positions along the root axis were deemed significant at 5% least significant difference (L.S.D).

5.4 Results

5.4.1 Uptake of NH_4^+, NO_3^- and H^+ ions in wheat cv. Revenue

5.4.1.1 Ammonium (NH_4^+)

There were significant differences (P = 0.001) between different positions along the root axis at 20 µM and 1 mM during normoxia and hypoxia (Figure 5.1). An efflux of NH_4^+ at 20 µM under normoxia was observed across all positions measured on the root axis. The efflux was more significant in the mature zone compared with the elongation zone, with an average of -11 and -3 µmol m^{-2} s^{-1}, respectively. At 1 mM, there was an influx of NH_4^+ across all positions on the root axis. The highest intake was registered at 2.5 mm from the root tip with an average of 141 µmol m^{-2} s^{-1}. The mature zone had an average intake of 41 µmol m^{-2} s^{-1} across all positions.

During hypoxia, there was an efflux of NH_4^+ at 20 µM for most positions on the root axis. The highest efflux occurred at 20 mm from the root tip at -20 µmol m^{-2} s^{-1}. At 1 mM, an uptake of NH_4^+ was observed across all positions in the elongation and mature zones. There were significant differences in NH_4^+ uptake between both zones. The overall average uptake of NH_4^+ was 46 and 321 µmol m^{-2} s^{-1} for the elongation and mature zone, respectively. The highest uptake occurred at 50 mm from the root tip at 553 µmol m^{-2} s^{-1}.

At 20 µM, hypoxic plants experienced reduced efflux of the NH_4^+ ions across the whole root compared with the control. Conversely, hypoxia increased NH_4^+ uptake across the whole root at 1 mM by almost three-fold of the control. A total uptake of 1423 µmol m^{-2} s^{-1} was observed compared with 501 µmol m^{-2} s^{-1} of the control.

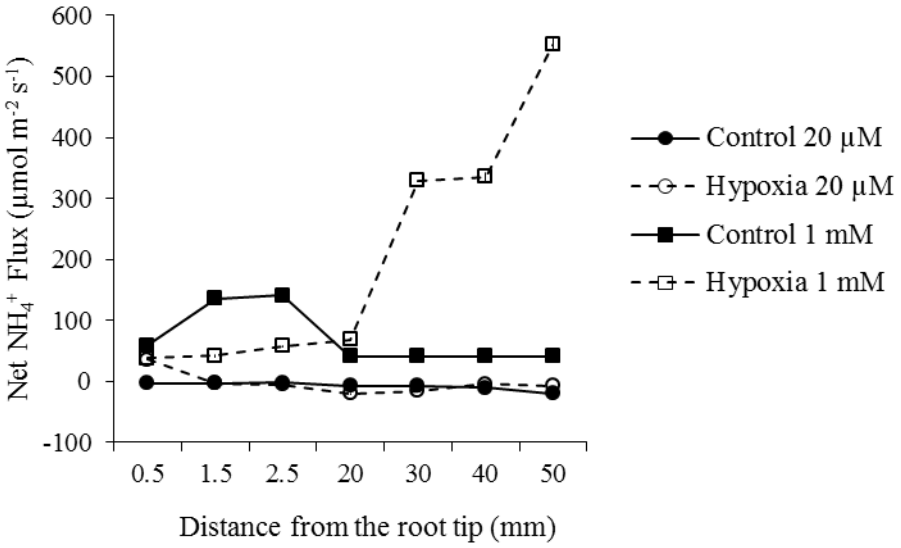

Figure 5.1: NH_4^+ uptake in wheat cv. Revenue under normoxia and hypoxia. Bars indicate s.e. (*n* = 5 individual plants). Note that in some cases, the bars are smaller than the symbols.

5.4.1.2 Nitrate (NO_3^-)

The uptake of NO_3^- varied significantly ($P = 0.001$) between different positions under normoxia and hypoxia at 20 µM and 1 mM (Figure 5.2). At 20 µM under normoxia, there was an efflux of NO_3^- ions in the elongation zone. The gradual increase in the efflux peaked at 2.5 mm from the root tip with an average of -62 µmol m^{-2} s^{-1}. On the contrary, there was an influx of NO_3^- ions across all positions in the mature zone with an overall average uptake of 60 µmol m^{-2} s^{-1}. At 1 mM, NO_3^- uptake occurred across different positions along the root axis. The elongation zone had a constant uptake rate of 81 µmol m^{-2} s^{-1} across all positions. However, the mature zone had a higher uptake than the elongation zone across all positions with an overall average of 110 µmol m^{-2} s^{-1}.

At 20 µM during hypoxia, there was an influx of NO_3^- in the elongation zone and at 20 mm from the root tip in the mature zone. The influx steadily decreased from 83 µmol m^{-2} s^{-1} at 0.5 mm from the root tip to 4 µmol m^{-2} s^{-1}, at 20 mm from the root tip. The continued decline resulted in efflux of NO_3^- ions for the remaining positions in the mature zone. At 1 mM, uptake of NO_3^- was observed across all positions along the root axis. The highest uptake at an average of 270 µmol m^{-2} s^{-1} occurred at 2.5 mm from the root tip. The uptake of NO_3^- increased steadily in the elongation zone and then markedly decreased in the mature zone where it maintained a relatively constant rate of 190 µmol m^{-2} s^{-1}.

Hypoxia increased the uptake of NO_3^- at 20 µM and 1 mM across the whole root. At 20 µM, the overall uptake of NO_3^- was 111 µmol m^{-2} s^{-1} under hypoxia compared with 74 µmol m^{-2} s^{-1} of the control. At 1 mM, NO_3^- uptake under hypoxia increased two-fold that of the control. A total uptake of 1361 µmol m^{-2} s^{-1} was observed compared with 684 µmol m^{-2} s^{-1} of the control.

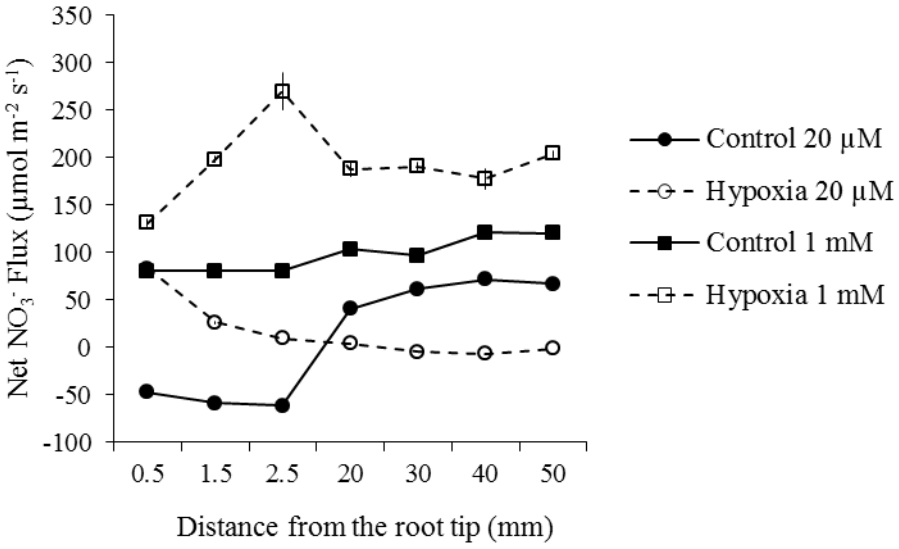

Figure 5.2: NO_3^- uptake in wheat cv. Revenue under normoxia and hypoxia. Bars indicate s.e. (*n* = 5 individual plants). Note that in some cases, the bars are smaller than the symbols.

5.4.1.3 Proton (H^+)

At 20 µM under normoxia, minimal efflux of the H^+ occurred in the elongation zone with an overall average of -1 µmol m^{-2} s^{-1}. Significant efflux occurred in the maturation zone with the highest efflux occurring at 30 mm from the root tip at an average of -111 µmol m^{-2} s^{-1} (Figure 5.3). Interestingly, at 1 mM there was an uptake of H^+ in the elongation zone at rate of 4 µmol m^{-2} s^{-1} whilst the mature zone had an efflux for all the four positions with the highest efflux occurring at 20 mm from the root tip at -57 µmol m^{-2} s^{-1}.

At 20 µM under hypoxia, there was an efflux of H^+ ions along the root axis; the average efflux was -2 and -1 µmol m^{-2} s^{-1} for the elongation and mature zone, respectively. Conversely, at 1 mM, there was an uptake of H^+ across all positions along the root axis maintained at a fairly constant rate of 0.4 µmol m^{-2} s^{-1}.

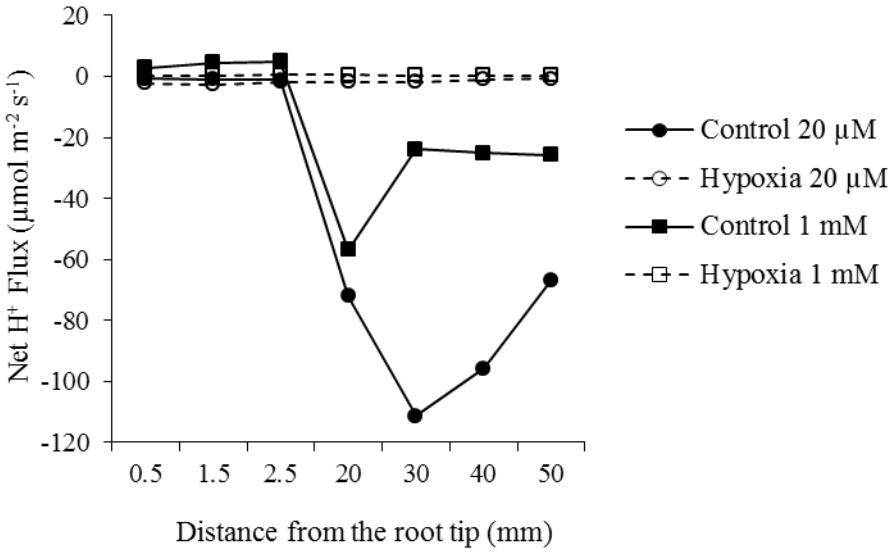

Figure 5.3: H^+ uptake in wheat cv. Revenue under normoxia and hypoxia. Bars indicate s.e. (*n* = 5 individual plants). Note that in some cases, the bars are smaller than the symbols.

5.4.2 Uptake of NH_4^+, NO_3^- and H^+ ions in barley cv. Naso Nijo

5.4.2.1 Ammonium (NH_4^+)

Significant variations ($P = 0.001$) in NH_4^+ efflux occurred across all positions along the root axis during normoxia at 20 µM. The efflux was higher in the mature zone with an average of -128 µmol m^{-2} s^{-1} compared with -64 µmol m^{-2} s^{-1} of the elongation zone. Significant efflux occurred at 40 and 50 mm from the root tip with an average of -180 and -175 µmol m^{-2} s^{-1}, respectively, which was more than double the efflux at 20 and 30 mm from the root tip. At 1 mM, NH_4^+ efflux increased steadily across all positions along the root axis from -98 to -211 µmol m^{-2} s^{-1} at 0.5 and 50 mm from the root tip, respectively (Figure 5.4). The mature zone had a higher efflux than the elongation zone.

During hypoxia, NH_4^+ uptake occurred in the elongation zone with an average of 175 µmol m^{-2} s^{-1} at 0.5 and 1.5 mm from the root tip, which declined markedly at 2.5 mm from

the root tip at 20 µM. The steady decline continued into the mature zone resulting in an efflux of NH_4^+ throughout the mature region at an average of -34 µmol m^{-2} s^{-1} (Figure 5.4). At 1 mM, NH_4^+ uptake occurred across all positions along the root axis characterised with significant differences between different positions (P = 0.001). In the elongation zone, the highest and lowest uptake occurred at 0.5 and 1.5 mm from the root tip, respectively. In the mature region, significant NH_4^+ uptake occurred at 30 mm from the root tip with 176 µmol m^{-2} s^{-1} followed by a marked decline at 40 and 50 mm from the root tip.

Hypoxia increased the overall uptake of NH_4^+ along the root axis at 20 µM with 255 µmol m^{-2} s^{-1} compared with -703 µmol m^{-2} s^{-1} of the control. Similarly, at 1 mM, hypoxia significantly increased NH_4^+ uptake across the whole root with 1070 µmol m^{-2} s^{-1} compared with -1028 µmol m^{-2} s^{-1} of the control.

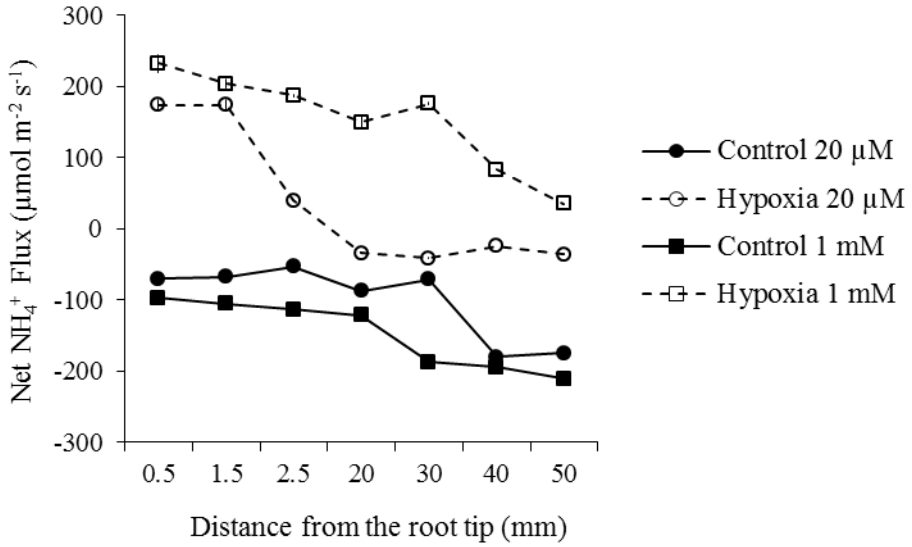

Figure 5.4: NH_4^+ uptake in barley cv. Naso Nijo under normoxia and hypoxia. Bars indicate s.e. (n = 5 individual plants). Note that in some cases, the bars are smaller than the symbols.

5.4.2.2 Nitrate (NO_3^-)

The pattern of NO_3^- uptake was similar at both 20 µM and 1 mM under normoxia (Figure 5.5). It was characterised by NO_3^- efflux in the elongation zone and NO_3^- uptake in the mature zone. The average efflux was -45 and -113 µmol m^{-2} s^{-1} at 20 µM and 1 mM, respectively. At 20 µM, NO_3^- uptake increased gradually in the mature zone from 10 µmol m^{-2} s^{-1} at the apical 20 mm to 41 µmol m^{-2} s^{-1}, at 50 mm from the root tip. At 1 mM, NO_3^- uptake significantly increased from 80 µmol m^{-2} s^{-1} at 20 mm from the root tip to 199 µmol m^{-2} s^{-1}, at 30 mm from the root tip. Thereafter, a marked decline was observed at 40 mm from the root tip followed by a slight increase at 50 mm from the root tip.

At 20 µM during hypoxia, the NO_3^- ion experienced minimal efflux at different positions along the root axis with the exception of positions at 0.5 and 50 mm from the root tip, which had an influx of 13 and 6 µmol m^{-2} s^{-1} respectively. There were no significant differences between positions with NO_3^- efflux ($P > 0.05$). At 1 mM, significant differences in NO_3^- uptake occurred across all positions along the root axis ($P = 0.001$). The elongation zone registered the highest uptake at an average of 16 µmol m^{-2} s^{-1} compared with the mature zone at 9 µmol m^{-2} s^{-1}. In the mature zone, NO_3^- uptake declined gradually from 17 µmol m^{-2} s^{-1} at 20 mm to 1 µmol m^{-2} s^{-1} at 50 mm from the root tip.

Hypoxia significantly increased total NO_3^- uptake across the whole root at 20 µM with 19 µmol m^{-2} s^{-1} compared with -47 µmol m^{-2} s^{-1} of the control. However, at 1 mM, hypoxia decreased overall NO_3^- uptake by 71%.

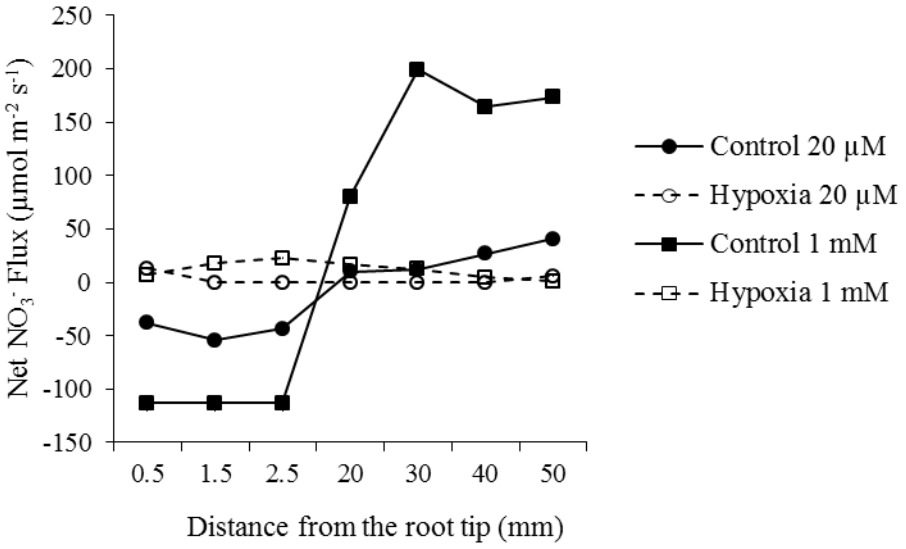

Figure 5.5: NO_3^- uptake in barley cv. Naso Nijo under normoxia and hypoxia. Bars indicate s.e. (*n* = 5 individual plants). Note that in some cases, the bars are smaller than the symbols.

5.4.2.2 Proton (H^+)

At 20 µM under normoxia, minimal efflux of the H^+ occurred across all positions along the root axis. There were significant differences (*P* = 0.001) between different positions and the average efflux was higher in the mature zone than the elongation zone. A similar pattern of H^+ efflux was observed at 1 mM (Figure 5.6).

Under hypoxia, there was an efflux of H^+ ions along the root axis at 20 µM. The average efflux was -1 µmol m^{-2} s^{-1} for both the elongation and mature zones. Interestingly at 1 mM, there was an uptake of H^+ across all positions along the root axis. The uptake in the elongation and mature zones was maintained at a fairly constant rate of 1 µmol m^{-2} s^{-1} (Figure 5.6).

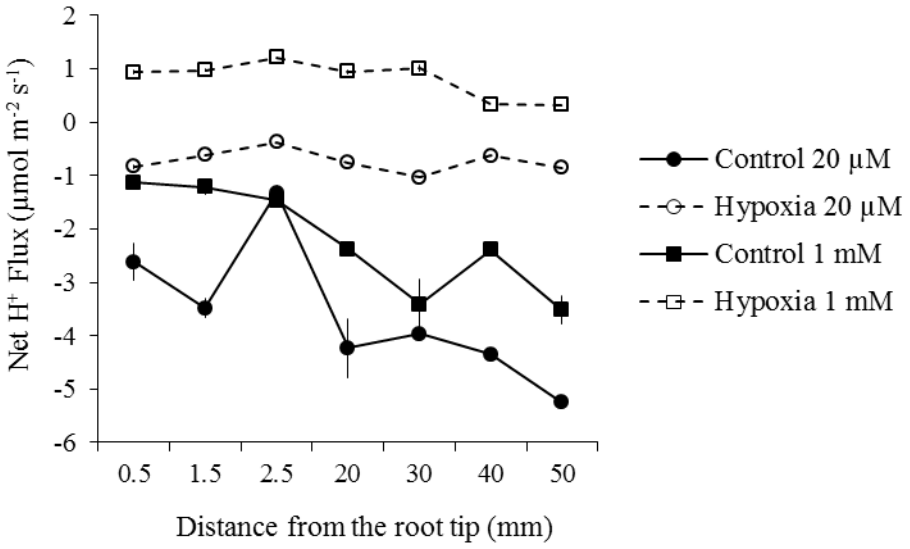

Figure 5.6: H^+ uptake in barley cv. Naso Nijo under normoxia and hypoxia. Bars indicate s.e. (*n* = 5 individual plants). Note that in some cases, the bars are smaller than the symbols.

In wheat, the total uptake of NO_3^- was significantly higher than NH_4^+ at 20 µM during hypoxia. However, at 1 mM the total uptake of NH_4^+ was higher than NO_3^-. For barley, the total NH_4^+ uptake was significantly higher than NO_3^- at 20 µM and 1 mM during hypoxia. Ammonium and NO_3^- had a total uptake of 255 and 19 µmol m^{-2} s^{-1} at 20 µM respectively. At 1 mM, the total uptake was 1070 and 82 µmol m^{-2} s^{-1} for NH_4^+ and NO_3^-, respectively. The total N uptake was higher in wheat, 2876 µmol m^{-2} s^{-1} than barley, 1424 µmol m^{-2} s^{-1} and the net fluxes of NH_4^+ and NO_3^- ions at different positions along the root axis were higher at 1 mM than at 20 µM for both wheat and barley varieties under normoxia and hypoxia. Overall, hypoxia increased N uptake compared with the control. In wheat cv. Revenue, the total N uptake was 1206 and 2876 µmol m^{-2} s^{-1} for the normoxia and hypoxia treatments, respectively whilst in barley cv. Naso Nijo, the total N uptake was -1501 and 1424 µmol m^{-2} s^{-1} for the normoxia and hypoxia treatments, respectively.

5.5 Discussion

Results from the present study and those of Pang et al. (2006) suggest that hypoxic conditions trigger mechanisms that mediate ion uptake resulting in higher uptake rates particularly at high concentrations. Predominantly net ion uptake has been reported to rise proportionally with external ion concentration (Forde & Clarkson 1999; Glass 2003); however, N acquisition during hypoxia is enhanced by the induction of the high-affinity transport system (HATS) (Glass et al. 2002; Feng et al. 2011; von Wirén et al. 2000). The HATS allows N uptake from low external concentrations (Glass et al. 2002; von Wirén et al. 1997), such as flooded paddy soils (Feng et al. 2011). The HATS also plays a significant role in retrieving the NH_4^+ lost from epidermal and cortical cells by efflux (Forde & Clarkson 1999) and contains cis-elements vital for NO_3^- uptake (Feng et al. 2011).

The preference for NH_4^+ over NO_3^- observed in this study under hypoxia could be attributed to some of the following factors. Firstly, the NO_3^- is often used as an alternative electron acceptor during respiration allowing the electron transport system (ETS) and oxidative phosphorylation to continue during hypoxia (Colmer 2015; Drew 1991). Secondly, the NO_3^- is reduced to nitrite (NO_2) by nitrate reductase (NR) and the NO_2 is further reduced to NH_4^+ by nitrite reductase (NiR) making it the dominant form of N under anaerobic conditions (Colmer 2015; Feng et al. 2011; Yan et al. 2011). Thirdly, the metabolic cost of NH_4^+ absorption and assimilation is lower than NO_3^- (Arnon 1937; von Wirén et al. 2000; Salsac et al. 1987), which accelerates NH_4^+ uptake. Nonetheless, NH_4^+ uptake is associated with the acidification of the rhizosphere, which alters membrane permeability and restricts NO_3^- uptake (Minotti et al. 1969; Newman 2001; Taylor & Bloom 1998). High rates of NH_4^+ influx also decrease the proton driving force that is vital for NO_3^- transport (Colmer & Bloom 1998) and excessive uptake of NH_4^+ due to

denitrification during hypoxia can cause plants to develop the NH_4^+ toxicity syndrome (Tylova-Munzarova et al. 2005), which results in restricted growth. Moreover, NH_4^+ can be converted to NO_3^- especially when radial oxygen loss (ROL) occurs in the rhizosphere (Colmer 2015), which possibly explains the higher total NO_3^- uptake under normoxia.

NH_4^+ and NO_3^- uptake in wheat and barley followed a similar pattern characterised by significant variations among different positions along the root axis. This implies that net fluxes of NH_4^+ and NO_3^- are often influenced by the metabolic state of localized groups of cells as suggested by Henriksen et al. (1992), who observed similar findings in roots of 7-day-old barley (*Hordeum vulgare* L. cv Prato) seedlings. Earlier studies by Henriksen et al. (1990), also showed that ion uptake varied both with position along the root axis and time. Besides, differential sensitivity of barley root tissues to hypoxia has been reported for other ions like K^+ by Pang et al. (2006). Restricted ion uptake particularly in the mature zone is often attributed to the presence of sclerenchymatous fibres, which tend to restrict nutrient uptake (Colmer & Bloom 1998), compared with the elongation zone, which is younger and more permeable to nutrients. The efflux of NO_3^- and NH_4^+ to the external media is a component of their net uptake. NH_4^+ efflux from the root elongation zone is linked with an inhibitory effect of NH_4^+ on primary root development, largely through repression of cell elongation (Xu et al. 2012) while NO_3^- efflux has been linked to a NO_3^- excretion transporter (*NAXT1*), which exhibits passive low-affinity NO_3^- efflux transport activity (Segonzac et al. 2007; Xu et al. 2012).

The variations in net H^+ flux observed in this study could be attributed to the differential uptake and assimilation of NH_4^+ and NO_3^- that generates a local variation in net H^+ extrusion (Taylor & Bloom 1998). Active efflux of H^+ ions to an extracellular sink helps counter intracellular acidity caused by excess H^+ generated in the root cell cytoplasm

(Raven & Smith 1976). This maintains electroneutrality during transport and regulates cytoplasmic pH (Henriksen et al. 1992). Nonetheless, H^+ can leak into the growing cells causing an influx of H^+ as observed at 1 mM during hypoxia for both wheat and barley (Taylor & Bloom 1998; Weisenseel et al. 1979). The evident genotypic differences between wheat and barley could be associated to the fact that wheat is more tolerant to hypoxic conditions than barley (Zhou 2010). Consistently, barley cv. Naso Nijo is said to be more sensitive to hypoxia (Pang et al. 2004) than other barley varieties. Despite the significant variations in NH_4^+, NO_3^- and H^+ flux patterns between different positions along the root axis at 20 µM and 1 mM NH_4NO_3 during normoxia and hypoxia in both wheat and barley, there was limited variation between individual plant roots (replicates) used in the experiment for both varieties. This limited variation is possibly due to the thorough processes and procedures taken during seedling growth and preparation of root samples for ion flux measurements as detailed in the materials and methods section of this chapter.

The uptake of NH_4^+ and NO_3^- under normoxic or aerobic conditions has been widely discussed (Xu et al. 2012). However, N uptake under limiting conditions such as waterlogging or hypoxia has received little attention. For instance Kiba and Krapp (2016), underscore the importance of further research in understanding N uptake under N-limited conditions or heterogeneous supply. The authors further highlight key research gaps that warrant further investigations including the polar localization of macronutrient transporters. Such knowledge is indispensable in helping us understand the mechanisms mediating N uptake under hypoxia. The research findings showed that the uptake of NH_4^+ and NO_3^- in selected wheat and barley varieties under hypoxia varies significantly along the root axis, including within the elongation and mature zones. Whilst previous research has shown nutrient uptake to increase with ion concentration under aerobic conditions (Glass 2003), the study findings showed that even under hypoxic conditions, there is an

up-regulation of mechanisms mediating N uptake, which results in higher N uptake rates. Further studies elucidating the physiological and molecular mechanisms behind this phenomenon will give an in depth understanding of N uptake under anaerobic conditions. The results also supported the proposed hypothesis that the uptake of NH_4^+, NO_3^- and H^+ will significantly vary along the root axis and between genotypes, and the uptake of NH_4^+ will be significantly higher than NO_3^-.

5.6 Conclusion

The uptake and efflux of NH_4^+, NO_3^- and H^+ ions varied significantly between different positions along the root axis for both wheat and barley. The fluxes of NH_4^+ and NO_3^- ions at different distances from the root tip were higher at 1 mM than at 20 µM for both wheat and barley under normoxia and hypoxia. Hypoxia increased N uptake for both wheat and barley and there was a preference for NH_4^+ over NO_3^-. Genotypic differences between wheat and barley were evident, with wheat having the highest uptake of both NH_4^+ and NO_3^- for the control and hypoxia at 20 µM and 1 mM. The findings highlight the dynamic process of N uptake during hypoxia, which is characterised by significant variations over relatively short distances and periods of time. Hypoxic conditions also seem to trigger mechanisms that mediate N uptake resulting in higher uptake rates.

Chapter Six: General Discussion

Waterlogging is a major abiotic constraint to cereal production in the HRZ of Australia (Acuña et al. 2011). It is estimated to cause yield losses of *ca*. 30-50% (Zhou 2010), and adversely affects plant growth and development (Pang et al. 2004), through reduced availability and uptake of essential nutrients (Pang et al. 2007; Pang et al. 2004). The research findings indicated that waterlogging significantly decreases plant growth and development in wheat and barley. Waterlogging significantly decreased the above-ground dry matter (AGDM) of selected wheat and barley varieties under controlled glass house conditions. However, plants have different physiological and morphological modifications such as aerenchyma formation, elongation of the internodes and development of numerous adventitious roots at the shoot base to counteract the adverse effects of waterlogging (Colmer & Voesenek 2009). These modifications differ between genotypes (Setter et al. 1999), with some genotypes showing an early onset of waterlogging tolerance mechanisms more than others (Zhou 2010). The study findings showed that, wheat varieties: Revenue, Tennant and Brennan are more tolerant to waterlogging than Mackellar and barley varieties, TX9425 and Naso Nijo. TX9425 was however, less sensitive to waterlogging than Naso Nijo.

6.1 Nitrogen uptake under hypoxic conditions

Under hypoxic conditions, NH_4^+ and NO_3^- uptake in wheat cv. Revenue and barley cv. Naso Nijo was characterised by significant variations among different positions along the root axis. Spatial and temporal variations in NH_4^+ and NO_3^- uptake along the root axis were also observed by Henriksen et al. (1992). This differential sensitivity of wheat and barley roots to hypoxia is often associated with the metabolic state of the localised cells and morphogical composition of the different root zones. The laboratory studies further

suggested that hypoxic conditions trigger mechanisms that mediate ion uptake resulting in higher uptake rates particularly at high concentrations. While net ion uptake has been reported to rise proportionally with external ion concentration (Glass 2003), N uptake during hypoxia is enhanced by the induction of the high-affinity transport system (HATS) (Feng et al. 2011). The plants also showed preference for NH_4^+ over NO_3^- during hypoxia. Much as NH_4^+ is the most abundant form of N under waterlogged conditions, NH_4^+ uptake is accelerated by the denitrification of NO_3^- to nitrite by nitrate reductase and consequently to NH_4^+ by nitrite reductase. NH_4^+ also has a lower metabolic cost of absorption and assimilation compared with NO_3^- in addition to its inhibitory role in NO_3^- uptake.

6.2 Role of N fertiliser application in ameliorating the adverse effects of waterlogging

The application of N fertilisers plays a significant role in improving plant growth and development under waterlogged conditions (Pang et al. 2007; Swarup & Sharma 1993). Applied N improves leaf photosynthetic capacity, shoot and root growth and enhances the production of adventitious roots (Pang et al. 2007). In our studies, under controlled glass house conditions, it was observed that N fertiliser application can improve plant growth and development during waterlogging and recovery. This improvement in plant growth could be attributed to the significant effect N fertiliser has on vegetative growth particularly tillering, canopy size and duration, and resultant biomass. Differences between N treatments were also evident for most of the parameters measured, with the CRF generally performing better than conventional urea. CRFs can release N over an extended period of time, which sustains N supply throughout the duration of the experiment whilst urea is often susceptible to leaching and denitrification particularly during waterlogging.

Under field conditions, N fertiliser application improved tiller number, ear number and AGDM for the different irrigation regimes at GS61. Similarly, at GS92, tiller number, ear

number and grain yield were responsive to N fertiliser application. The urea treatments had an average of 8 t/ha for both the rainfed and irrigated, and 5.3 t/ha for the waterlogged. In a separate study, average wheat yields of 8 t/ha were reported for the HRZ of south-eastern Australia (Acuña et al. 2011). Such yields particularly under rainfed conditions could be associated with the cool-temperate climate in the region receiving over 550 mm of rainfall annually. The CRF had the highest amount of grain yield for all irrigation regimes with 9.2 t/ha, 9.4 t/ha and 6.8 t/ha for the rainfed, irrigated and waterlogged, respectively. The CRF also improved NUE by 17% and 27% more than single- and split-applied urea, respectively under waterlogged conditions; however, there were no significant statistical differences between the urea treatments and the CRF. CRFs can release N over an extended period of time, which sustains N supply during crop growth and maximize NUE by synchronizing N release with crop demand (Chen et al. 2008). Conventional urea on the other hand, often supplied as $CO(NH_2)_2$ is highy soluble and readily provides N, which increases crop vegetative growth (Pang et al. 2014), and offsets the adverse effects of waterlogging. Furthermore, top-dressing urea after waterlogging boosts secondary tiller production and increases canopy duration thus increasing the photosynthetic capacity and overall productivity of the plant. However, top-dressing urea after waterlogging can be difficult under field conditions as the soil moisture content is often too high for efficient mechanization.

Noteworthy, besides fertiliser type, seasonality has a significant effect on soil N availability (Cassman et al. 2002). Seasonality impacts on the balance between N immobilization and mineralization as mediated by the soil microbes, contributions from applied organic and inorganic N sources and losses from the plant-available pool (Cassman et al. 2002). These factors may affect N release from CRFs and influence

nutrient supply relative to crop demand. In addition, N uptake is chiefly dependent on plant demand, which is influenced by the crop growth stage.

6.3 Economic analysis of fertiliser use in broadacre cropping under different irrigation regimes

Gross margins defined as the financial difference between returns received from sales of produce and the variable costs associated with producing that produce (DPIPWE 2017), were calculated based on the prevailing wheat grain price of AU$220 /t (DPIPWE 2017), taking into account the variable costs of the different fertilisers and seed used. The fungicide treated seed was purchased at AU$35.45 /25kg while urea and the CRF (Agrocote) were purchased at AU$30.15 /25kg and AU$55 /25kg, respectively. Gross margins allow comparisons to be made between enterprises (DPIPWE 2017), which fosters informed decision making particularly when adopting new technologies associated with highly variable commodity prices. Consequently, a sensitivity analysis to the different fertiliser prices for both urea and the CRF was performed under different irrigation regimes to further support farmers with informed decision making (Pannell 1997). The sensitivity analysis was performed using Microsoft Excel 2010 (What-If Analysis) with fertiliser prices varying from 15.1 to 75.4 AU$/25kg for urea and from 27.5 to 137.5 AU$/25kg for the CRF. The fertiliser base price was 1.21 and 2.2 AU$/kg for urea and the CRF, respectively and the rest of the input data were kept constant.

The results indicated that the CRF generated the highest gross margin under rainfed conditions while single-applied urea had the highest gross margin under irrigated and waterlogged conditions (Table 6.1). There were no significant statistical differences between single- and split-applied urea and CRF for all irrigation regimes. Interestingly, under irrigated conditions, the nil N treatment had a relatively close gross margin of 1,243

AU$/ha compared with the average of 1,369 AU$/ha of both urea treatments and the CRF. Overall, waterlogging decreased the productivity of wheat by nearly 50% compared with respective N treatments of the rainfed and irrigated regimes.

Table 6. 1: Gross margins (AU$/ha) on fertiliser use under different irrigation regimes

Irrigation regime	Rainfed	Irrigated	Waterlogged
Nitrogen treatment			
Nil N	657ab	1,243ab	613a
Single-applied urea	1,272ab	1,412b	805ab
Split-applied urea	1,309ab	1,324ab	676ab
CRF	1,346ab	1,371ab	797ab

*Treatments followed by the same letter are not significantly different ($P > 0.05$) (l.s.d: 447).

A sensitivity analysis to fertiliser price varying from 15.1 to 75.4 AU$/25kg for urea and from 27.5 to 137.5 AU$/25kg for the CRF under different irrigation regimes was performed with the rest of the input data constant. Using a base price of 1.21 and 2.2 AU$/kg for urea and the CRF, respectively, the results showed that increasing fertiliser price leads to a linear decline in the gross margins obtained for both urea and the CRF under different irrigation regimes (Table 6.2). Given that N fertilisers are now constituting the single largest variable cost for most grain growers (DPIPWE 2017), it is important for farmers to maximise profits by taking into account the relative fertiliser and commodity prices (Abadi & Farre 2015), in addition to agronomic information (Sadras et al. 2016), when deciding the appropriate source N fertiliser. For instance, from an economic point of view, the use of CRFs, which are often of higher price than conventional urea, would not be advisable in areas prone to waterlogging. However, since waterlogging is often transient and intermittent, use of CRFs with the appropriate release period could be a viable option. Furthermore, with the intensification of cropping systems and increased

reliance of N fertilisers, the challenge for growers to supply the right amount of N at the right time and place to meet crop demand and optimise yield quality, without oversupplying N and reducing profit can be achieved by farmers making informed guesses based on soil, seasonal and economic indicators (Sadras et al. 2016).

Table 6. 2: Gross margins (AU$/ha) for increasing prices under different regimes

Fertiliser	Urea			CRF		
Fertiliser price	Rainfed	Irrigated	Waterlogged	Rainfed	Irrigated	Waterlogged
Standard price	1,291	1,368	741	1,346	1,371	797
N price x 0.5	1,408	1,485	858	1,601	1,626	1,052
N price x 1.5	1,173	1,250	623	1,096	1,122	548
N price x 2	1,055	1,132	505	844	870	295
N price x 2.5	943	1,021	394	592	617	43

*The standard fertiliser prices were 30.15 and 55 AU$/25kg for urea and CRF (Agrocote), respectively.

6.4 Conclusions and recommendations

Although some research has been conducted in the different HRZs of Australia to understand the impact of waterlogging on wheat and barley growth under field conditions (Robertson et al. 2009; Setter & Waters 2003; Setter et al. 2009), most work in Tasmania has been conducted under controlled or glasshouse conditions (Colmer et al. 2001; Falakboland et al. 2017; Malik et al. 2002; Pang et al. 2007; Pang et al. 2004). The field study conducted corroborated other studies and literature (Acuña et al. 2011), indicating that waterlogging is one the major abiotic constraints to cereal production in the region. The research also explored the potential of CRFs in mitigating the adverse effects of waterlogging and their use in broadacre cropping compared with conventional urea. The CRF improved wheat and barley growth under different irrigation regimes, however, the trend was not statistically different compared with conventional urea. Furthermore, the

research also investigated the uptake of NH_4^+ and NO_3^- ions along the root axis under hypoxia; highlighting the significant variations between the different root zones and need for understanding the processes and mechanisms involved in the up-regulation of N uptake during hypoxia as these can be exploited by plant breeders to develop waterlogging tolerant and nutrient-use efficient crops.

All in all, the findings are experimental evidence that N fertiliser application can alleviate the adverse effects of waterlogging in cereals. The timing of N application and source are important. Applying the full amount of the required fertiliser at sowing helps plants to withstand the adverse effects of transient and intermittent waterlogging through enhanced vegetative growth. Top-dressing urea after waterlogging and relieve of waterlogging stress enhances plant growth during recovery. Using CRFs may improve cereal growth and NUE through sustained N supply though there might be no significant yield advantage over conventional urea to warrant investment depending on the prevailing fertiliser price, which is highly variable. With the increased availablity of information and the different types of enhanced-efficient fertilisers, cereal producers can make informed decisions on fertiliser use given the current harsh climate change effects. Furthermore, the findings highlight the dynamic process of N uptake during hypoxia, which is characterised by significant variations over relatively short distances and periods of time. Hypoxic conditions also seem to trigger mechanisms that mediate N uptake resulting in higher uptake rates. The genetic variability in tolerance to waterlogging observed between different cultivars and genotypes can be exploited by breeders in their efforts to breed for more resource-use efficient crops.

In response to the key hypotheses this research sought to investigate, the following deductions are proposed:

(a) Sensitivity to waterlogging varies between wheat and barley and within individual genotypes;

(b) Timing of N fertiliser application and source of applied N are crucial in alleviating the adverse effects of waterlogging in cereals;

(c) Controlled-release fertilisers can improve yield and NUE in cereals under waterlogged conditions though might not be significantly different from conventional urea and;

(d) Temporal and spatial variations occur in NH_4^+ and NO_3^- uptake along the root axis and there is preference for NH_4^+ over NO_3^- during hypoxia.

Knowledge gaps that still warrant future studies include:

(a) The evaluation of different enhanced-efficient fertilisers (EEFs) for their potential in broadacre cropping. There are different forms of EEFs available on the global market for instance: nitrification inhibitors (NIs), polymer-coated fertilizers (PCFs)/CRFs and urease inhibitors (UIs) (Chen et al. 2008). While the potential of different CRFs for example: Agrocote/Agromaster, Environmentally Smart Nitrogen (ESN) and Meister has been evaluated with promising potential to increase crop yields and improve NUE, the potential of nitrification inhibitors particularly in the HRZs prone to waterlogging remains inconclusive. Yet these inhibitors are said to be effective in flood prone areas or waterlogged soils where N loss due to leaching and denitrification is a common occurrence (Akiyama et al. 2010; Chen et al. 2008). This could be achieved through controlled studies, field experiments and simulation modelling, testing different scenarios.

(b) The evaluation of different CRFs and nitrification inhibitors with different longevity or release periods and release rates to assess the optimal time for N application rather than a single basal application at planting as the efficacy of these

fertilisers is dependent on the fertilizer formulation, crop type, soil climate and management factors (Chen et al. 2008). For instance, Shoji et al. (2001) observed that ~20% of the CRF N can remain within the fertilizer pellets at harvest if a fertilizer with the appropriate formulation or release rate is not selected. Furthermore, the development of better CRFs is vital in the improvement of the efficacy of these fertilizers and improvement of NUE. The research findings have shown that CRFs can improve crop growth and NUE under different irrigation regimes. However, understanding the underlying processes and mechanisms influencing the efficacy of these fertilisers under different farming practices is paramount for the development of better CRFs.

(c) Unravelling the molecular and physiological mechanisms responsible for inducing N uptake during hypoxia. The study findings showed that under hypoxic conditions, there is an increase in N uptake, which could be attributed to an upregulation of the mechanisms mediating N uptake resulting in higher N uptake rates. The HATS has been suggested to be activated under N limiting conditions (Feng et al. 2011) and the expression of *AtNRT2.1, AtNRT2.2, AtNRT2.4* and *AtNRT2.5* also occurs in N deprived roots (Kotur & Glass 2015), however, it remains to be investigated, if these same processes or mechanisms are responsible for N uptake under hypoxic conditions where there is a combination of limited N and O_2 availability.

7.0 References

Abadi, A & Farre, I 2015, 'A simple framework for profitable fertiliser use under risk and soil constraints', in *Proceedings of the 17th Australian Society of Agronomy Conference*, 20th – 24th September, Hobart, Tasmania, Australia.

ABARES 2017, *Agricultural commodities and trade data* Australian Government, Department of Agriculture and Water Resources, Australian Bureau of Agricultural and Resource Economics and Sciences (ABARES), viewed 24th July 2017, <http://www.agriculture.gov.au/abares/research-topics/agricultural-commodities/agricultural-commodities-trade-data#crops>.

Acuña, TB, Dean, G & Riffkin, P 2011, 'Constraints to achieving high potential yield of wheat in a temperate, high-rainfall environment in south-eastern Australia', *Crop and Pasture Science*, vol. 62, no. 2, pp. 125-136.

Adams, C, Frantz, J & Bugbee, B 2013, 'Macro-and micronutrient-release characteristics of three polymer-coated fertilizers: Theory and measurements', *Journal of Plant Nutrition and Soil Science*, vol. 176, no. 1, pp. 76-88.

Ahmed, F, Rafii, M, Ismail, MR, Juraimi, AS, Rahim, H, Asfaliza, R & Latif, M 2012, 'Waterlogging tolerance of crops: breeding, mechanism of tolerance, molecular approaches, and future prospects', *BioMed Research International*, vol. 2013, pp. 1-10.

Akiyama, H, Yan, X & Yagi, K 2010, 'Evaluation of effectiveness of enhanced-efficiency fertilizers as mitigation options for N_2O and NO emissions from agricultural soils: meta-analysis', *Global Change Biology*, vol. 16, no. 6, pp. 1837-1846.

Alexandre, A, Silva, J, Bouma, TJ & Santos, R 2011, 'Inorganic nitrogen uptake kinetics and whole-plant nitrogen budget in the seagrass Zostera noltii', *Journal of Experimental Marine Biology and Ecology*, vol. 401, no. 1, pp. 7-12.

Almagro, A, Lin, SH & Tsay, YF 2008, 'Characterization of the Arabidopsis nitrate transporter NRT1. 6 reveals a role of nitrate in early embryo development', *The Plant Cell*, vol. 20, no. 12, pp. 3289-3299.

Alva, AK 1992, 'Differential leaching of nutrients from soluble vs. controlled-release fertilizers', *Environmental Management*, vol. 16, no. 6, pp. 769-776.

Anderson, W 1985, 'Grain yield responses of barley and durum wheat to split nitrogen applications under rainfed conditions in a Mediterranean environment', *Field Crops Research*, vol. 12, pp. 191-202.

Armstrong, W & Drew, M 2002, 'Root growth and metabolism under oxygen deficiency', *Plant roots: the hidden half*, vol. 3, pp. 729-761.

Arnon, DI 1937, 'Ammonium and nitrate nitrogen nutrition of barley at different seasons in relation to hydrogen-ion concentration, manganese, copper, and oxygen supply', *Soil Science*, vol. 44, no. 2, pp. 91-122.

Ashraf, MA 2012, 'Waterlogging stress in plants: A review', *African Journal of Agricultural Research*, vol. 7, no. 13, pp. 1976-1981.

Aslam, M, Travis, RL & Huffaker, RC 1992, 'Comparative kinetics and reciprocal inhibition of nitrate and nitrite uptake in roots of uninduced and induced barley (*Hordeum vulgare* L.) seedlings', *Plant Physiology*, vol. 99, no. 3, pp. 1124-1133.

Asseng, S, Turner, N & Keating, B 2001, 'Analysis of water-and nitrogen-use efficiency of wheat in a Mediterranean climate', *Plant and Soil*, vol. 233, no. 1, pp. 127-143.

Bailey-Serres, J & Voesenek, LACJ 2008, 'Flooding stress: acclimations and genetic diversity', *Annual Review of Plant Biology*, vol. 59, pp. 313-339.

Bakker, D, Hamilton, G, Houlbrooke, D & Spann, C 2005, 'The effect of raised beds on soil structure, waterlogging, and productivity on duplex soils in Western Australia', *Soil Research*, vol. 43, no. 5, pp. 575-585.

Bakker, D, Hamilton, G, Houlbrooke, D, Spann, C & Van Burgel, A 2007, 'Productivity of crops grown on raised beds on duplex soils prone to waterlogging in Western Australia', *Animal Production Science*, vol. 47, no. 11, pp. 1368-1376.

Beddington, J 2010, 'Food security: contributions from science to a new and greener revolution', *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 365, no. 1537, pp. 61-71.

Belford, R 1981, 'Response of winter wheat to prolonged waterlogging under outdoor conditions', *Journal of Agricultural Science, Cambridge*, vol. 97, pp. 557-568.

Belford, R, Dracup, M & Tennant, D 1992, 'Limitations to growth and yield of cereal and lupin crops on duplex soils', *Animal Production Science*, vol. 32, no. 7, pp. 929-945.

BOM 2015, Bureau of Meteorology, viewed 10th February 2015, <http://www.bom.gov.au/climate/averages/tables/cw_091306.shtml>.

Botella, MA, Cerdá, A & Lips, S 1994, 'Kinetics of NO_3^- and NH_4^+ uptake by wheat seedlings. Effect of salinity and nitrogen source', *Journal of Plant Physiology*, vol. 144, no. 1, pp. 53-57.

Bradley, PM & Morris, JT 1990, 'Influence of oxygen and sulfide concentration on nitrogen uptake kinetics in *Spartina alterniflora*', *Ecology*, vol. 71, no. 1, pp. 282-287.

Breteler, H & Siegerist, M 1984, 'Effect of ammonium on nitrate utilization by roots of dwarf bean', *Plant Physiology*, vol. 75, no. 4, pp. 1099-1103.

Burney, JA, Davis, SJ & Lobell, DB 2010, 'Greenhouse gas mitigation by agricultural intensification', *Proceedings of the National Academy of Sciences*, vol. 107, no. 26, pp. 12052-12057.

Buwalda, F & Greenway, H 1989, 'Nitrogen uptake and growth of wheat during O_2 deficiency in root media containing NO_3^- only, or NO_3^- plus NH_4^+', *New Phytologist*, vol. 111, no. 2, pp. 161-166.

Buwalda, F, Barrett-Lennard, E, Greenway, H & Davies, B 1988, 'Effects of growing wheat in hypoxic nutrient solutions and of subsequent transfer to aerated solutions. II. Concentrations and uptake of nutrients and sodium in shoots and roots', *Functional Plant Biology*, vol. 15, no. 4, pp. 599-612.

Cai, J, Jiang, D, Liu, F, Dai, T & Cao, W 2011, 'Effects of split nitrogen fertilization on post-anthesis photoassimilates, nitrogen use efficiency and grain yield in malting barley', *Acta Agriculturae Scandinavica, Section B-Soil and Plant Science*, vol. 61, no. 5, pp. 410-420.

Cai, J, Jiang, D, Wollenweber, B, Dai, T & Cao, W 2012, 'Effects of nitrogen application rate on dry matter redistribution, grain yield, nitrogen use efficiency and photosynthesis in

malting barley', *Acta Agriculturae Scandinavica, Section B-Soil and Plant Science*, vol. 62, no. 5, pp. 410-419.

Cassman, KG, Dobermann, A & Walters, DT 2002, 'Agroecosystems, Nitrogen-use Efficiency, and Nitrogen Management', *AMBIO: A Journal of the Human Environment*, vol. 31, no. 2, pp. 132-140.

Chen, D, Suter, H, Islam, A, Edis, R, Freney, J & Walker, C 2008, 'Prospects of improving efficiency of fertiliser nitrogen in Australian agriculture: a review of enhanced efficiency fertilisers', *Soil Research*, vol. 46, no. 4, pp. 289-301.

Chittleborough, D 1992, 'Formation and pedology of duplex soils', *Animal Production Science*, vol. 32, no. 7, pp. 815-825.

Chiu, C-C, Lin, C-S, Hsia, A-P, Su, R-C, Lin, H-L & Tsay, Y-F 2004, 'Mutation of a nitrate transporter, AtNRT1: 4, results in a reduced petiole nitrate content and altered leaf development', *Plant and Cell Physiology*, vol. 45, no. 9, pp. 1139-1148.

Chopin, F, Orsel, M, Dorbe, M-F, Chardon, F, Truong, H-N, Miller, AJ, Krapp, A & Daniel-Vedele, F 2007, 'The Arabidopsis ATNRT2. 7 nitrate transporter controls nitrate content in seeds', *The Plant Cell*, vol. 19, no. 5, pp. 1590-1602.

Collaku, A & Harrison, S 2005, 'Heritability of waterlogging tolerance in wheat', *Crop Science*, vol. 45, no. 2, pp. 722-727.

Collaku, A & Harrison, SA 2002, 'Losses in wheat due to waterlogging', *Crop Science*, vol. 42, no. 2, pp. 444-450.

Colmer, T & Voesenek, L 2009, 'Flooding tolerance: suites of plant traits in variable environments', *Functional Plant Biology*, vol. 36, no. 8, pp. 665-681.

Colmer, TD & Bloom, AJ 1998, 'A comparison of NH_4^+ and NO_3^- net fluxes along roots of rice and maize', *Plant, Cell & Environment*, vol. 21, no. 2, pp. 240-246.

Colmer, TD & Greenway, H 2011, 'Ion transport in seminal and adventitious roots of cereals during O_2 deficiency', *Journal of Experimental Botany*, vol. 62, no. 1, pp. 39-57.

Colmer, TD, Atwell, BJ, Ismail, AM, Pedersen, O, Shabala, S, Sorrell, B & Voesenek, LACJ 2015, 'Waterlogging and submergence', in TD Colmer (ed.), *Plants in Action: a resource for teachers and students in plant science*, second edn, Australian Society of Plant Scientists, http://plantsinaction.science.uq.edu.au.

Colmer, TD, Lambers, H & Schortemeyer, M 2001, 'Changes in physiological and morphological traits of roots and shoots of wheat in response to different depths of waterlogging', *Functional Plant Biology*, vol. 28, no. 11, pp. 1121-1131.

Condon, A, Richards, R, Rebetzke, G & Farquhar, G 2002, 'Improving intrinsic water-use efficiency and crop yield', *Crop Science*, vol. 42, no. 1, pp. 122-131.

Condon, AG & Giunta, F 2003, 'Yield response of restricted-tillering wheat to transient waterlogging on duplex soils', *Crop and Pasture Science*, vol. 54, no. 10, pp. 957-967.

Cotching, W, Lynch, S & Kidd, D 2009, 'Dominant soil orders in Tasmania: distribution and selected properties', *Soil Research*, vol. 47, no. 5, pp. 537-548.

Cox, J & McFarlane, D 1995, 'The causes of waterlogging in shallow soils and their drainage in southwestern Australia', *Journal of Hydrology*, vol. 167, no. 1, pp. 175-194.

Crawford, NM & Glass, ADM 1998, 'Molecular and physiological aspects of nitrate uptake in plants', *Trends in Plant Science*, vol. 3, no. 10, pp. 389-395.

Crawford, R & Braendle, R 1996, 'Oxygen deprivation stress in a changing environment', *Journal of Experimental Botany*, vol. 47, no. 2, pp. 145-159.

Criddle, RS, Ward, MR & Huffaker, RC 1988, 'Nitrogen uptake by wheat seedlings, interactive effects of four nitrogen sources: NO_3^-, NO_2^-, NH_4^+, and urea', *Plant Physiology*, vol. 86, no. 1, pp. 166-175.

Daniel-Vedele, F, Filleur, S & Caboche, M 1998, 'Nitrate transport: a key step in nitrate assimilation', *Current Opinion in Plant Biology*, vol. 1, no. 3, pp. 235-239.

Deane-Drummond, CE & Glass, AD 1983, 'Short term studies of nitrate uptake into barley plants using ion-specific electrodes and $^{36}ClO_3^-$ I. Control of net uptake by NO_3^- efflux', *Plant Physiology*, vol. 73, no. 1, pp. 100-104.

Di, H & Cameron, K 2002, 'Nitrate leaching in temperate agroecosystems: sources, factors and mitigating strategies', *Nutrient Cycling in Agroecosystems*, vol. 64, no. 3, pp. 237-256.

Dinnes, DL, Karlen, DL, Jaynes, DB, Kaspar, TC, Hatfield, JL, Colvin, TS & Cambardella, CA 2002, 'Nitrogen management strategies to reduce nitrate leaching in tile-drained Midwestern soils', *Agronomy Journal*, vol. 94, no. 1, pp. 153-171.

Dobermann, A & Cassman, KG 2005, 'Cereal area and nitrogen use efficiency are drivers of future nitrogen fertiliser consumption', *Science in China Series C: Life Sciences*, vol. 48, pp. 745-758.

DPIPWE 2017, *Farm Business Planning Tools*, Department of Primary Industries, Parks, Water and Environment (DPIPWE), viewed 12th September 2017, <http://dpipwe.tas.gov.au/agriculture/investing-in-irrigation/farm-business-planning-tools>.

Drew, M & Sisworo, E 1977, 'Early effects of flooding on nitrogen deficiency and leaf chlorosis in barley', *New Phytologist*, vol. 79, no. 3, pp. 567-571.

Drew, MC 1988, 'Effects of flooding and oxygen deficiency on plant mineral nutrition', *Advances in Plant Nutrition.*, vol. 3, pp. 115-159.

Drew, MC 1991, 'Oxygen deficiency in the root environment and plant mineral nutrition', *Plant Life under Oxygen Deprivation*, pp. 303-316.

Drew, MC 1997, 'Oxygen deficiency and root metabolism: injury and acclimation under hypoxia and anoxia', *Annual Review of Plant Biology*, vol. 48, no. 1, pp. 223-250.

Elzenga, J & van Veen, H 2010, 'Waterlogging and plant nutrient uptake', in S Shabala & S Mancuso (eds), *Waterlogging signalling and tolerance in plants*, Springer-Verlag, Berlin, pp. 23-32.

Ercoli, L, Lulli, L, Mariotti, M, Masoni, A & Arduini, I 2008, 'Post-anthesis dry matter and nitrogen dynamics in durum wheat as affected by nitrogen supply and soil water availability', *European Journal of Agronomy*, vol. 28, no. 2, pp. 138-147.

Evans, R, Fausey, N, Skaggs, R & Schilfgaarde, J 1999, 'Effects of inadequate drainage on crop growth and yield', *Agricultural Drainage, Agronomy Monograph*, no. 38, pp. 13-54.

Falakboland, Z, Zhou, M, Zeng, F, Kiani-Pouya, A, Shabala, L & Shabala, S 2017, 'Plant ionic relation and whole-plant physiological responses to waterlogging, salinity and their combination in barley', *Functional Plant Biology*, vol. 44, no. 9, pp. 941-953.

Fan, S-C, Lin, C-S, Hsu, P-K, Lin, S-H & Tsay, Y-F 2009, 'The Arabidopsis nitrate transporter NRT1. 7, expressed in phloem, is responsible for source-to-sink remobilization of nitrate', *The Plant Cell*, vol. 21, no. 9, pp. 2750-2761.

Fang, XZ, Tian, WH, Liu, XX, Lin, XY, Jin, CW & Zheng, SJ 2016, 'Alleviation of proton toxicity by nitrate uptake specifically depends on nitrate transporter 1.1 in Arabidopsis', *New Phytologist*, vol. 211, no. 1, pp. 149-158.

FAO 2008, *Current world fertiliser trends and outlook to 2011/12*, Food and Agriculture Organisation of the United Nations, viewed 21.12.2012, <ftp://ftp.fao.org/agl/agll/docs/cwfto11.pdf>.

FAO 2010, *Current world fertiliser trends and outlook to 2014* Food and Agriculture Organisation of the United Nations viewed 21.12.2012, <ftp://ftp.fao.org/ag/agp/docs/cwfto14.pdf>.

Farooq, M, Hussain, M & Siddique, KHM 2014, 'Drought Stress in Wheat during Flowering and Grain-filling Periods', *Critical Reviews in Plant Sciences*, vol. 33, no. 4, pp. 331-349.

Feng, H, Yan, M, Fan, X, Li, B, Shen, Q, Miller, AJ & Xu, G 2011, 'Spatial expression and regulation of rice high-affinity nitrate transporters by nitrogen and carbon status', *Journal of Experimental Botany*, vol. 62, no. 7, pp. 2319-2332.

Fillery, I & McInnes, K 1992, 'Components of the fertiliser nitrogen balance for wheat production on duplex soils', *Animal Production Science*, vol. 32, no. 7, pp. 887-899.

Fischer, R 1993, 'Irrigated spring wheat and timing and amount of nitrogen fertilizer. II. Physiology of grain yield response', *Field Crops Research*, vol. 33, no. 1, pp. 57-80.

Fischer, RA, Byerlee, D & Edmeades, GO 2014, *Crop yields and global food security: will yield increase continue to feed the world?*, Australian Centre for International Agricultural Research, Canberra, Australia, <http://aciar.gov.au/publication/mn158>.

Fischer, RA, Howe, GN & Ibrahim, Z 1993, 'Irrigated spring wheat and timing and amount of nitrogen fertilizer. I. Grain yield and protein content', *Field Crops Research*, vol. 33, no. 1, pp. 37-56.

Fois, S, Motzo, R & Giunta, F 2009, 'The effect of nitrogenous fertiliser application on leaf traits in durum wheat in relation to grain yield and development', *Field Crops Research*, vol. 110, no. 1, pp. 69-75.

Forde, BG & Clarkson, DT 1999, 'Nitrate and ammonium nutrition of plants: physiological and molecular perspectives', *Advances in Botanical Research*, vol. 30, pp. 1-90.

Forde, BG 2000, 'Nitrate transporters in plants: structure, function and regulation', *Biochimica et Biophysica Acta (BBA) - Biomembranes*, vol. 1465, no. 1–2, pp. 219-235.

Freney, J, Smith, C & Mosier, A 1992, 'Effect of a new nitrification inhibitor (wax coated calcium carbide) on transformations and recovery of fertilizer nitrogen by irrigated wheat', *Nutrient Cycling in Agroecosystems*, vol. 32, no. 1, pp. 1-11.

Gandeza, AT & Shoji, S 1992, 'Properties of polyolefin-coated fertilizers', in S Shoji (ed.), *Controlled release fertilizers with polyolefin resin coating*, Konno Printing Co Ltd, Sendai, Japan, pp. 22-35.

Garnett, TP, Shabala, SN, Smethurst, PJ & Newman, IA 2001, 'Simultaneous measurement of ammonium, nitrate and proton fluxes along the length of eucalypt roots', *Plant and Soil*, vol. 236, no. 1, pp. 55-62.

Garnett, TP, Shabala, SN, Smethurst, PJ & Newman, IA 2003, 'Kinetics of ammonium and nitrate uptake by eucalypt roots and associated proton fluxes measured using ion selective microelectrodes', *Functional Plant Biology*, vol. 30, no. 11, pp. 1165-1176.

Gazzarrini, S, Lejay, L, Gojon, A, Ninnemann, O, Frommer, WB & von Wirén, N 1999, 'Three functional transporters for constitutive, diurnally regulated, and starvation-induced uptake of ammonium into Arabidopsis roots', *The Plant Cell*, vol. 11, no. 5, pp. 937-947.

Giles, J 2005, 'Nitrogen study fertilizes fears of pollution', *Nature*, vol. 433, no. 7028, pp. 791-791.

Gill, MB, Zeng, F, Shabala, L, Zhang, G, Fan, Y, Shabala, S & Zhou, M 2017, 'Cell-Based Phenotyping Reveals QTL for Membrane Potential Maintenance Associated with Hypoxia and Salinity Stress Tolerance in Barley', *Frontiers in Plant Science*, vol. 8, no. 1941.

Glass, A 1988, 'Nitrogen uptake by plant roots', *ISI Atlas of Science: Animal and Plant Sciences*, vol. 1, no. 2, pp. 151-156.

Glass, AD 2003, 'Nitrogen use efficiency of crop plants: physiological constraints upon nitrogen absorption', *Critical Reviews in Plant Sciences*, vol. 22, no. 5, pp. 453-470.

Glass, AD, Britto, DT, Kaiser, BN, Kinghorn, JR, Kronzucker, HJ, Kumar, A, Okamoto, M, Rawat, S, Siddiqi, M & Unkles, SE 2002, 'The regulation of nitrate and ammonium transport systems in plants', *Journal of Experimental Botany*, vol. 53, no. 370, pp. 855-864.

Global Market Insights Inc. 2017, *Controlled Release Fertilizers Market to Exceed $3.9 Billion by 2024*, viewed 12th September 2017, <http://www.prnewswire.com/news-releases/controlled-release-fertilizers-market-to-exceed-39-billion-by-2024-global-market-insights-inc-617418663.html>.

Goggin, DE & Colmer, TD 2007, 'Wheat genotypes show contrasting abilities to recover from anoxia in spite of similar anoxic carbohydrate metabolism', *Journal of Plant Physiology*, vol. 164, no. 12, pp. 1605-1611.

Gordon, W 2009, 'Starter fertilizer application method and composition in reduced-tillage corn production', *Better Crops with Plant Food*, vol. 2, no. 93, pp. 10-11.

Goyal, SS & Huffaker, RC 1986, 'The Uptake of NO_3^-, NO_2^-, and NH_4^+ by Intact Wheat (*Triticum aestivum*) Seedlings I. Induction and Kinetics of Transport Systems', *Plant Physiology*, vol. 82, no. 4, pp. 1051-1056.

Grant, C, Gauer, L, Bailey, L & Gehl, D 1991, 'Protein production and nitrogen utilization by barley cultivars in response to nitrogen fertilizer under varying moisture conditions', *Canadian Journal of Plant Science*, vol. 71, no. 4, pp. 997-1009.

GRDC 2011, *Tasmanian growers shoulder marketing challenge*, Grains Research and Development Corporation (GRDC), viewed 12th April 2016, <https://grdc.com.au/Media-Centre/Ground-Cover/Ground-Cover-Issue-92-May-June-2011/Tasmanian-growers-shoulder-marketing-challenge>.

Greenway, H, Armstrong, W & Colmer, TD 2006, 'Conditions leading to high CO_2 (> 5 KPa) in waterlogged–flooded soils and possible effects on root growth and metabolism', *Annals of Botany*, vol. 98, no. 1, pp. 9-32.

Grose, CJ & Moreton, RM 1996, *Land capability survey of Tasmania. South Esk Report.*, Department of Primary Industry and Fisheries (DPIF), Tasmania, Australia., Tasmania, Australia.

Hardie, MA, Doyle, RB, Cotching, WE & Lisson, S 2012, 'Subsurface Lateral Flow in Texture-Contrast (Duplex) Soils and Catchments with Shallow Bedrock', *Applied and Environmental Soil Science*, vol. 2012.

Harper, L, Langdale, G & Giddens, J 1987, 'Nitrogen cycling in a wheat crop: soil, plant, and aerial nitrogen transport', *Agronomy Journal*, vol. 79, no. 6, pp. 965-973.

Hauck, R 1985, 'Slow release and bio-inhibitor-amended nitrogen fertilizers', in O Engelstad (ed.), *Fertilizer Use Technology*, Soil Science Society of America, Madison, WI, pp. 293-322.

Hawkins, H-J & Lewis, O 1993, 'Effect of NaCl salinity, nitrogen form, calcium and potassium concentration on nitrogen uptake and kinetics in *Triticum aestivum* L. cv. Gamtoos', *New Phytologist*, vol. 124, no. 1, pp. 171-177.

Haynes, R 1990, 'Active ion uptake and maintenance of cation-anion balance: A critical examination of their role in regulating rhizosphere pH', *Plant and Soil*, vol. 126, no. 2, pp. 247-264.

Heffer, P 2009, *Assessment of fertiliser use by crop at the global level*, International Fertiliser Industry Association, viewed 12.11.2012, <html/Assessment-of-Fertiliser-Use-by-Crop-at-the-Global-Level-2006-07-2007-08. html2>.

Henriksen, GH, Bloom, AJ & Spanswick, RM 1990, 'Measurement of net fluxes of ammonium and nitrate at the surface of barley roots using ion-selective microelectrodes', *Plant Physiology*, vol. 93, no. 1, pp. 271-280.

Henriksen, GH, Raman, DR, Walker, LP & Spanswick, RM 1992, 'Measurement of net fluxes of ammonium and nitrate at the surface of barley roots using ion-selective microelectrodes II. Patterns of uptake along the root axis and evaluation of the microelectrode flux estimation technique', *Plant Physiology*, vol. 99, no. 2, pp. 734-747.

Hill, KJ, Santoso, A & England, MH 2009, 'Interannual Tasmanian rainfall variability associated with large-scale climate modes', *Journal of Climate*, vol. 22, no. 16, pp. 4383-4397.

Hillel, D 1980, *Fundamentals of soil physics*, Academic Press, New York.

Hirel, B, Le Gouis, J, Ney, B & Gallais, A 2007, 'The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches', *Journal of Experimental Botany*, vol. 58, no. 9, pp. 2369-2387.

Hoel, BO 1998, 'Use of a hand-held chlorophyll meter in winter wheat: Evaluation of different measuring positions on the leaves', *Acta Agriculturae Scandinavica B—Plant Soil Sciences*, vol. 48, no. 4, pp. 222-228.

Hossain, MA & Uddin, S 2011, 'Mechanisms of waterlogging tolerance in wheat: Morphological and metabolic adaptations under hypoxia or anoxia', *Australian Journal of Crop Science*, vol. 5, no. 9, pp. 1094-1101.

Huang, B, Johnson, JW, NeSmith, DS & Bridges, DC 1994a, 'Root and shoot growth of wheat genotypes in response to hypoxia and subsequent resumption of aeration', *Crop Science*, vol. 34, no. 6, pp. 1538-1544.

Huang, B, Johnson, JW, Nesmith, S & Bridges, DC 1994b, 'Growth, physiological and anatomical responses of two wheat genotypes to waterlogging and nutrient supply', *Journal of Experimental Botany*, vol. 45, no. 2, pp. 193-202.

Huang, N-C, Liu, K-H, Lo, H-J & Tsay, Y-F 1999, 'Cloning and functional characterization of an Arabidopsis nitrate transporter gene that encodes a constitutive component of low-affinity uptake', *The Plant Cell*, vol. 11, no. 8, pp. 1381-1392.

Huett, DO & Gogel, BJ 2000, 'Longevities and nitrogen, phosphorus, and potassium release patterns of polymer-coated controlled-release fertilizers at 30° C and 40° C', *Communications in Soil Science and Plant Analysis*, vol. 31, no. 7-8, pp. 959-973.

IHS Markit 2015, *Chemical Economics Handbook: Controlled- and Slow-Release Fertilizers* viewed 12th September 2017, <https://www.ihs.com/products/controlled-and-slow-release-chemical-economics-handbook.html>.

ImpactFertilisers 2018, viewed 24th April 2018, <http://www.impactfertilisers.com.au/products/specialty-fertilisers/agromaster/>.

Jarvis, S 1996, 'Future trends in nitrogen research', *Plant and Soil*, vol. 181, no. 1, pp. 47-56.

Jiang, J, Hu, Z, Sun, W & Huang, Y 2010, 'Nitrous oxide emissions from Chinese cropland fertilized with a range of slow-release nitrogen compounds', *Agriculture, Ecosystems and Environment*, vol. 135, no. 3, pp. 216-225.

Ju, XT, Xing, GX, Chen, XP, Zhang, SL, Zhang, LJ, Liu, XJ, Cui, ZL, Yin, B, Christie, P & Zhu, ZL 2009, 'Reducing environmental risk by improving N management in intensive Chinese agricultural systems', *Proceedings of the National Academy of Sciences*, vol. 106, no. 9, pp. 3041-3046.

Kassem, M & Al-Suker, A 2009, 'Effect of fertigation methods on productivity and nitrogen use efficiency for wheat and barley crops', *Irrigation and Drainage*, vol. 26, no. 2, pp. 866-885.

Kiba, T & Krapp, A 2016, 'Plant Nitrogen Acquisition Under Low Availability: Regulation of Uptake and Root Architecture', *Plant and Cell Physiology*, vol. 57, no. 4, pp. 707-714.

King, BJ, Siddiqi, MY, Ruth, TJ, Warner, RL & Glass, AD 1993, 'Feedback regulation of nitrate influx in barley roots by nitrate, nitrite, and ammonium', *Plant Physiology*, vol. 102, no. 4, pp. 1279-1286.

Kochba, M, Gambash, S & Avnimelech, Y 1990, 'Studies on slow release fertilizers: 1. Effects of temperature, soil moisture and water vapor pressure', *Soil Science*, vol. 149, no. 6, pp. 339-343.

Kotur, Z & Glass, AD 2015, 'A 150 kDa plasma membrane complex of AtNRT2. 5 and AtNAR2. 1 is the major contributor to constitutive high-affinity nitrate influx in Arabidopsis thaliana', *Plant, Cell & Environment*, vol. 38, no. 8, pp. 1490-1502.

Krishnakumari, M, Sharma, R & Balloli, S 2000, 'Effect of late application of nitrogen on yield and protein content of wheat', *Annals of Agricultural Research*, vol. 21, no. 2, pp. 288-291.

Kronzucker, H, Glass, A, Siddiqi, M & Kirk, G 2000, 'Comparative kinetic analysis of ammonium and nitrate acquisition by tropical lowland rice: implications for rice cultivation and yield potential', *New Phytologist*, vol. 145, no. 3, pp. 471-476.

Kronzucker, HJ, Glass, AD & Siddiqi, MY 1999, 'Inhibition of nitrate uptake by ammonium in barley. Analysis of component fluxes', *Plant Physiology*, vol. 120, no. 1, pp. 283-292.

Kronzucker, HJ, Kirk, GJ, Siddiqi, MY & Glass, AD 1998, 'Effects of hypoxia on $^{13}NH_4^+$ fluxes in rice roots kinetics and compartmental analysis', *Plant Physiology*, vol. 116, no. 2, pp. 581-587.

Kronzucker, HJ, Siddiqi, MY & Glass, AD 1995, 'Compartmentation and flux characteristics of nitrate in spruce', *Planta*, vol. 196, no. 4, pp. 674-682.

Ladha, JK, Pathak, H, J Krupnik, T, Six, J & van Kessel, C 2005, 'Efficiency of fertiliser nitrogen in cereal production: retrospects and prospects', *Advances in Agronomy*, vol. 87, pp. 85-156.

Lam, H-M, Coschigano, KT, Oliveira, IC, Melo-Oliveira, R & Coruzzi, GM 1996, 'The molecular-genetics of nitrogen assimilation into amino acids in higher plants', *Annual Review of Plant Physiology and Plant Molecular Biology*, vol. 47, no. 1, pp. 569-593.

Latiri-Souki, K, Nortcliff, S & Lawlor, DW 1998, 'Nitrogen fertilizer can increase dry matter, grain production and radiation and water use efficiencies for durum wheat under semi-arid conditions', *European Journal of Agronomy*, vol. 9, no. 1, pp. 21-34.

Lea, PJ & Azevedo, RA 2006, 'Nitrogen use efficiency. 1. Uptake of nitrogen from the soil', *Annals of Applied Biology*, vol. 149, no. 3, pp. 243-247.

Lezhneva, L, Kiba, T, Feria-Bourrellier, A-B, Lafouge, F, Boutet-Mercey, S, Zoufan, P, Sakakibara, H, Daniel-Vedele, F & Krapp, A 2014, 'The Arabidopsis nitrate transporter NRT2. 5 plays a role in nitrate acquisition and remobilization in nitrogen-starved plants', *The Plant Journal*, vol. 80, no. 2, pp. 230-241.

Li, J-Y, Fu, Y-L, Pike, SM, Bao, J, Tian, W, Zhang, Y, Chen, C-Z, Zhang, Y, Li, H-M & Huang, J 2010, 'The Arabidopsis nitrate transporter NRT1. 8 functions in nitrate removal from the xylem sap and mediates cadmium tolerance', *The Plant Cell*, vol. 22, no. 5, pp. 1633-1646.

Li, W, Wang, Y, Okamoto, M, Crawford, NM, Siddiqi, MY & Glass, AD 2007, 'Dissection of the AtNRT2. 1: AtNRT2. 2 inducible high-affinity nitrate transporter gene cluster', *Plant Physiology*, vol. 143, no. 1, pp. 425-433.

Li, X, Hu, C, Delgado, JA, Zhang, Y & Ouyang, Z 2007, 'Increased nitrogen use efficiencies as a key mitigation alternative to reduce nitrate leaching in north china plain', *Agricultural Water Management*, vol. 89, no. 1, pp. 137-147.

Lin, S-H, Kuo, H-F, Canivenc, G, Lin, C-S, Lepetit, M, Hsu, P-K, Tillard, P, Lin, H-L, Wang, Y-Y & Tsai, C-B 2008, 'Mutation of the Arabidopsis NRT1. 5 nitrate transporter causes defective root-to-shoot nitrate transport', *The Plant Cell*, vol. 20, no. 9, pp. 2514-2528.

Liu, J, You, L, Amini, M, Obersteiner, M, Herrero, M, Zehnder, AJB & Yang, H 2010, 'A high-resolution assessment on global nitrogen flows in cropland', *Proceedings of the National Academy of Sciences*, vol. 107, no. 17, pp. 8035-8040.

Liu, K-H & Tsay, Y-F 2003, 'Switching between the two action modes of the dual-affinity nitrate transporter CHL1 by phosphorylation', *The EMBO Journal*, vol. 22, no. 5, pp. 1005-1013.

López-Bellido, L, López-Bellido, RJ & López-Bellido, FJ 2006, 'Fertiliser nitrogen efficiency in durum wheat under rainfed Mediterranean conditions: Effect of split application', *Agronomy Journal*, vol. 98, no. 1, pp. 55-62.

López-Bellido, L, López-Bellido, RJ & Redondo, R 2005, 'Nitrogen efficiency in wheat under rainfed Mediterranean conditions as affected by split nitrogen application', *Field Crops Research*, vol. 94, no. 1, pp. 86-97.

Lubkowski, K & Grzmil, B 2007, 'Controlled release fertilisers', *Polish Journal of Chemical Technology*, vol. 9, no. 4, pp. 83-84.

Ludewig, U, Neuhäuser, B & Dynowski, M 2007, 'Molecular mechanisms of ammonium transport and accumulation in plants', *FEBS Letters*, vol. 581, no. 12, pp. 2301-2308.

Malik, AI, Colmer, TD, Lambers, H, Setter, TL & Schortemeyer, M 2002, 'Short-term waterlogging has long-term effects on the growth and physiology of wheat', *New Phytologist*, vol. 153, no. 2, pp. 225-236.

Malik, AI, Islam, AKMR & Colmer, TD 2011, 'Transfer of the barrier to radial oxygen loss in roots of *Hordeum marinum* to wheat (*Triticum aestivum*): evaluation of four *H. marinum*–wheat amphiploids', *New Phytologist*, vol. 190, no. 2, pp. 499-508.

Markets & Markets 2017, *Controlled-Release Fertilizers Market*, viewed 12th September 2017, <http://www.marketsandmarkets.com/PressReleases/controlled-release-fertilizers.asp>.

Marschner, P 2011, *Marschner's Mineral Nutrition of Higher Plants*, Academic Press.

Mascagni Jr, H & Sabbe, W 1991, 'Late spring nitrogen applications on wheat on a poorly drained soil', *Journal of Plant Nutrition*, vol. 14, no. 10, pp. 1091-1103.

Mathers, NJ, Nash, DM & Gangaiya, P 2007, 'Nitrogen and phosphorus exports from high rainfall zone cropping in Australia: Issues and opportunities for research', *Journal of Environmental Quality*, vol. 36, no. 6, pp. 1551-1562.

Matsunaga, R, Ito, O, Tobita, S, Rao, TP & Johansen, C 1994, 'Response of short-duration pigeonpea to nitrogen application after short-term waterlogging on a Vertisol', *Field Crops Research*, vol. 38, no. 3, pp. 167-174.

Maxwell, K & Johnson, GN 2000, 'Chlorophyll fluorescence-a practical guide', *Journal of Experimental Botany*, vol. 51, no. 345, pp. 659-668.

Mengel, K & Kirkby, E 2001, *Principles of Plant Nutrition*, Kluwer Academic Publishers, Netherlands, London.

Minotti, P, Williams, DC & Jackson, W 1969, 'Nitrate uptake by wheat as influenced by ammonium and other cations', *Crop Science*, vol. 9, no. 1, pp. 9-14.

Mohammed, YA, Kelly, J, Chim, BK, Rutto, E, Waldschmidt, K, Mullock, J, Torres, G, Desta, KG & Raun, W 2013, 'Nitrogen Fertiliser Management for Improved Grain Quality

and Yield in Winter Wheat in Oklahoma', *Journal of Plant Nutrition*, vol. 36, no. 5, pp. 749-761.

Moll, R, Kamprath, E & Jackson, W 1982, 'Analysis and interpretation of factors which contribute to efficiency of nitrogen utilization', *Agronomy Journal*, vol. 74, no. 3, pp. 562-564.

Morison, J, Baker, N, Mullineaux, P & Davies, W 2008, 'Improving water use in crop production', *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 363, no. 1491, pp. 639-658.

Morris, JT 1980, 'The nitrogen uptake kinetics of *Spartina alterniflora* in culture', *Ecology*, pp. 1114-1121.

Mosier, A, Duxbury, J, Freney, J, Heinemeyer, O & Minami, K 1996, 'Nitrous oxide emissions from agricultural fields: Assessment, measurement and mitigation', *Plant and Soil*, vol. 181, no. 1, pp. 95-108.

Mosier, A, Duxbury, J, Freney, J, Heinemeyer, O & Minami, K 1998, 'Assessing and mitigating N_2O emissions from agricultural soils', *Climatic Change*, vol. 40, no. 1, pp. 7-38.

Nacry, P, Bouguyon, E & Gojon, A 2013, 'Nitrogen acquisition by roots: physiological and developmental mechanisms ensuring plant adaptation to a fluctuating resource', *Plant and Soil*, vol. 370, no. 1-2, pp. 1-29.

Newman, IA 2001, 'Ion transport in roots: measurement of fluxes using ion-selective microelectrodes to characterize transporter function', *Plant, Cell & Environment*, vol. 24, no. 1, pp. 1-14.

Noellsch, A, Motavalli, P, Nelson, K & Kitchen, N 2009, 'Corn response to conventional and slow-release nitrogen fertilizers across a claypan landscape', *Agronomy Journal*, vol. 101, no. 3, pp. 607-614.

O'Brien, JA, Vega, A, Bouguyon, E, Krouk, G, Gojon, A, Coruzzi, G & Gutiérrez, RA 2016, 'Nitrate transport, sensing, and responses in plants', *Molecular Plant*, vol. 9, no. 6, pp. 837-856.

Oertli, J 1980, 'Controlled-release fertilizers', *Nutrient Cycling in Agroecosystems*, vol. 1, no. 2, pp. 103-123.

Okamoto, M, Kumar, A, Li, W, Wang, Y, Siddiqi, MY, Crawford, NM & Glass, AD 2006, 'High-affinity nitrate transport in roots of Arabidopsis depends on expression of the NAR2-like gene AtNRT3. 1', *Plant Physiology*, vol. 140, no. 3, pp. 1036-1046.

Okamoto, M, Vidmar, JJ & Glass, AD 2003, 'Regulation of *NRT1* and *NRT2* gene families of *Arabidopsis thaliana*: responses to nitrate provision', *Plant and Cell Physiology*, vol. 44, no. 3, pp. 304-317.

Olfs, HW, Blankenau, K, Brentrup, F, Jasper, J, Link, A & Lammel, J 2005, 'Soil-and plant-based nitrogen-fertiliser recommendations in arable farming', *Journal of Plant Nutrition and Soil Science*, vol. 168, no. 4, pp. 414-431.

Orsel, M, Chopin, F, Leleu, O, Smith, SJ, Krapp, A, Daniel-Vedele, F & Miller, AJ 2006, 'Characterization of a two-component high-affinity nitrate uptake system in Arabidopsis. Physiology and protein-protein interaction', *Plant Physiology*, vol. 142, no. 3, pp. 1304-1317.

Orsel, M, Filleur, S, Fraisier, V & Daniel-Vedele, F 2002a, 'Nitrate transport in plants: which gene and which control?', *Journal of Experimental Botany*, vol. 53, no. 370, pp. 825-833.

Orsel, M, Krapp, A & Daniel-Vedele, F 2002b, 'Analysis of the NRT2 nitrate transporter family in Arabidopsis. Structure and gene expression', *Plant Physiology*, vol. 129, no. 2, pp. 886-896.

Ortiz-Monasterio, R, Sayre, K, Rajaram, S & McMahon, M 1997, 'Genetic progress in wheat yield and nitrogen use efficiency under four nitrogen rates', *Crop Science*, vol. 37, no. 3, pp. 898-904.

Pan, WL, Jackson, WA & Moll, RH 1985, 'Nitrate uptake and partitioning by corn root systems. Differential effects of ammonium among genotypes and stages of root development', *Plant Physiology*, vol. 77, no. 3, pp. 560-566.

Pang, J, Palta, JA, Rebetzke, GJ & Milroy, SP 2014, 'Wheat genotypes with high early vigour accumulate more nitrogen and have higher photosynthetic nitrogen use efficiency during early growth', *Functional Plant Biology*, vol. 41, no. 2, pp. 215-222.

Pang, J, Ross, J, Zhou, M, Mendham, N & Shabala, S 2007, 'Amelioration of detrimental effects of waterlogging by foliar nutrient sprays in barley', *Functional Plant Biology*, vol. 34, no. 3, pp. 221-227.

Pang, J, Zhou, M, Mendham, N & Shabala, S 2004, 'Growth and physiological responses of six barley genotypes to waterlogging and subsequent recovery', *Crop and Pasture Science*, vol. 55, no. 8, pp. 895-906.

Pang, JY, Newman, I, Mendham, N, Zhou, M & Shabala, S 2006, 'Microelectrode ion and O_2 fluxes measurements reveal differential sensitivity of barley root tissues to hypoxia', *Plant, Cell and Environment*, vol. 29, no. 6, pp. 1107-1121.

Pannell, DJ 1997, 'Sensitivity analysis of normative economic models: theoretical framework and practical strategies', *Agricultural Economics*, vol. 16, no. 2, pp. 139-152.

Parelle, J, Dreyer, E & Brendel, O 2010, 'Genetic variability and determinism of adaptation of plants to soil waterlogging', in S Shabala & S Mancuso (eds), *Waterlogging signalling and tolerance in plants* Springer-Verlag, Berlin, pp. 241-265.

Parent, C, Capelli, N, Berger, A, Crèvecoeur, M & Dat, JF 2008, 'An overview of plant responses to soil waterlogging', *Plant Stress*, vol. 2, no. 1, pp. 20-27.

Perata, P & Alpi, A 1993, 'Plant responses to anaerobiosis', *Plant Science*, vol. 93, no. 1, pp. 1-17.

Phillips, S & Mullins, G 2004, 'Foliar burn and wheat grain yield responses following topdress-applied nitrogen and sulfur fertilizers', *Journal of Plant Nutrition*, vol. 27, no. 5, pp. 921-930.

Plett, D, Toubia, J, Garnett, T, Tester, M, Kaiser, BN & Baumann, U 2010, 'Dichotomy in the *NRT* gene families of dicots and grass species', *PLoS One*, vol. 5, no. 12, pp. e15289-e15289.

Ponnamperuma, F 1972, *The chemistry of submerged soils*, vol. 24, Academic Press NY and London.

Rane, J, Ishitani, M & Rao, I 2011, 'Response of crop plants to nitrogen stress: opportunities to increase nitrogen use efficiency', in M Pessarakli (ed.), *Hand book of plant*

crop stress, 3rd edn, CRC Press, Taylor and Francis Group, Boca Raton, Florida, pp. 339-359.

Raun, WR & Johnson, GV 1999, 'Improving nitrogen use efficiency for cereal production', *Agronomy Journal*, vol. 91, no. 3, pp. 357-363.

Raven, J & Smith, F 1976, 'Nitrogen assimilation and transport in vascular land plants in relation to intracellular pH regulation', *New Phytologist*, pp. 415-431.

Rhee, KC 2001, 'Determination of Total Nitrogen', in *Current Protocols in Food Analytical Chemistry*, John Wiley & Sons, Inc., <http://dx.doi.org/10.1002/0471142913.fab0102s00>.

Riffkin, P & Sylvester-Bradley, R 2008, 'A wheat ideotype for the High rainfall Zone of south-west Victoria ', in *Proceedings of the 14th Australian Agronomy Conference*, 21st - 25th September, Adelaide, South Australia, Australia.

Robertson, D, Zhang, H, Palta, JA, Colmer, T & Turner, NC 2009, 'Waterlogging affects the growth, development of tillers, and yield of wheat through a severe, but transient, N deficiency', *Crop and Pasture Science*, vol. 60, no. 6, pp. 578-586.

Rufty, TW, Jackson, WA & Raper, CD 1982, 'Inhibition of nitrate assimilation in roots in the presence of ammonium: the moderating influence of potassium', *Journal of Experimental Botany*, vol. 33, no. 6, pp. 1122-1137.

Sadras, V & Angus, J 2006, 'Benchmarking water-use efficiency of rainfed wheat in dry environments', *Crop and Pasture Science*, vol. 57, no. 8, pp. 847-856.

Sadras, VO & Lawson, C 2013, 'Nitrogen and water-use efficiency of Australian wheat varieties released between 1958 and 2007', *European Journal of Agronomy*, vol. 46, pp. 34-41.

Sadras, VO, Hayman, PT, Rodriguez, D, Monjardino, M, Bielich, M, Unkovich, M, Mudge, B & Wang, E 2016, 'Interactions between water and nitrogen in Australian cropping systems: physiological, agronomic, economic, breeding and modelling perspectives', *Crop and Pasture Science*, vol. 67, no. 10, pp. 1019-1053.

Sairam, R, Kumutha, D, Ezhilmathi, K, Deshmukh, P & Srivastava, G 2008, 'Physiology and biochemistry of waterlogging tolerance in plants', *Biologia Plantarum*, vol. 52, no. 3, pp. 401-412.

Salsac, L, Chaillou, S, Lesaint, C & Jolivet, E 1987, 'Nitrate and ammonium nutrition in plants [organic anion, ion accumulation, osmolarity]', *Plant Physiology and Biochemistry*, vol. 25, no. 6, pp. 805-812.

Schenk, M 1996, 'Regulation of nitrogen uptake on the whole plant level', *Plant and Soil*, vol. 181, no. 1, pp. 131-137.

Schrader, L, Domska, D, Jung, P & Peterson, L 1972, 'Uptake and assimilation of ammonium-N and nitrate-N and their influence on the growth of corn (*Zea mays* L.)', *Agronomy Journal*, vol. 64, no. 5, pp. 690-695.

Seednet 2018, *https://www.seednet.com.au/products/wheat*, viewed 24th April 2018.

Segonzac, C, Boyer, J-C, Ipotesi, E, Szponarski, W, Tillard, P, Touraine, B, Sommerer, N, Rossignol, M & Gibrat, R 2007, 'Nitrate efflux at the root plasma membrane: identification of an Arabidopsis excretion transporter', *The Plant Cell*, vol. 19, no. 11, pp. 3760-3777.

Setter, T & Waters, I 2003, 'Review of prospects for germplasm improvement for waterlogging tolerance in wheat, barley and oats', *Plant and Soil*, vol. 253, no. 1, pp. 1-34.

Setter, T, Burgess, P, Waters, I & Kuo, J 1999, 'Genetic diversity of barley and wheat for waterlogging tolerance in Western Australia', in *Proceedings of the 9th Australian Barley Technical Symposium. Melbourne, Australian Barley Technical Symposium Inc*.

Setter, T, Waters, I, Sharma, S, Singh, K, Kulshreshtha, N, Yaduvanshi, N, Ram, P, Singh, B, Rane, J & McDonald, G 2009, 'Review of wheat improvement for waterlogging tolerance in Australia and India: the importance of anaerobiosis and element toxicities associated with different soils', *Annals of Botany*, vol. 103, no. 2, pp. 221-235.

Seward, E 1984, 'Slow-release nitrogen fertilizers', in R Hauck (ed.), *Nitrogen in crop production*, Madison, WI, pp. 195-206.

Shabala, S, Demidchik, V, Shabala, L, Cuin, TA, Smith, SJ, Miller, AJ, Davies, JM & Newman, IA 2006, 'Extracellular Ca^{2+} ameliorates NaCl-induced K^+ loss from Arabidopsis root and leaf cells by controlling plasma membrane K^+-permeable channels', *Plant Physiology*, vol. 141, no. 4, pp. 1653-1665.

Shangguan, Z, Shao, M & Dyckmans, J 2000a, 'Nitrogen nutrition and water stress effects on leaf photosynthetic gas exchange and water use efficiency in winter wheat', *Environmental and Experimental Botany*, vol. 44, no. 2, pp. 141-149.

Shangguan, Z, Shao, M & Dyckmans, J 2000b, 'Effects of nitrogen nutrition and water deficit on net photosynthetic rate and chlorophyll fluorescence in winter wheat', *Journal of Plant Physiology*, vol. 156, no. 1, pp. 46-51.

Sharma, P, Jha, A & Dubey, R 2011, 'Oxidative stress and antioxidative defense systems in plants growing under abiotic stresses', in M Pessarakli (ed.), *Hand book of plat crop stress*, 3rd edn, CRC Press, Taylor and Francis Group, Boca Raton, Florida, pp. 115-116.

Shaviv, A & Mikkelsen, R 1993, 'Controlled-release fertilisers to increase efficiency of nutrient use and minimize environmental degradation - A review', *Nutrient Cycling in Agroecosystems*, vol. 35, no. 1, pp. 1-12.

Shaviv, A 2001, 'Advances in controlled-release fertilizers', *Advances in Agronomy*, vol. Volume 71, pp. 1-49.

Shoji, S, Delgado, J, Mosier, A & Miura, Y 2001, 'Use of controlled release fertilisers and nitrification inhibitors to increase nitrogen use efficiency and to conserve air and water quality', *Communications in Soil Science and Plant Analysis*, vol. 32, no. 7-8, pp. 1051-1070.

Siddiqi, MY, Glass, ADM, Ruth, TJ & Rufty, TW 1990, 'Studies of the uptake of nitrate in barley I. Kinetics of $^{13}NO_3^-$ influx', *Plant Physiology*, vol. 93, no. 4, pp. 1426-1432.

Sieling, K, Schröder, H, Finck, M & Hanus, H 1998, 'Yield, N uptake, and apparent N-use efficiency of winter wheat and winter barley grown in different cropping systems', *The Journal of Agricultural Science*, vol. 131, no. 04, pp. 375-387.

Sinclair, T & Rufty, T 2012, 'Nitrogen and water resources commonly limit crop yield increases, not necessarily plant genetics', *Global Food Security*, vol. 1, pp. 94-98.

Smith, P, Martino, D, Cai, Z, Gwary, D, Janzen, H, Kumar, P, McCarl, B, Ogle, S, O'Mara, F, Rice, C, Scholes, B & Sirotenko, O 2007, 'Agriculture', in B Metz, OR Davidson, PR Bosch, R Dave & LA Meyer (eds), *In Climate Change 2007: Mitigation.*

Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 499-540.

Stewart, T, Norton, S, Madanat, J & Stewart, H 2008, 'Global Food Crisis: Urgent Need and Emerging Solutions', *Sustainable Development Law and Policy*, vol. 9, pp. 31-35,71-72.

Strong, W 1982, 'Effect of late application of nitrogen on the yield and protein content of wheat', *Animal Production Science*, vol. 22, no. 115, pp. 54-61.

Sundaram, B, Feitz, A, Caritat, P, Plazinska, Ad, Brodie, R, Coram, J & Ransley, T 2009, *Groundwater sampling and analysis - a field guide*, 2009/27 95pp, Geoscience Australia, Canberra, ACT.

Swarup, A & Sharma, D 1993, 'Influence of top-dressed nitrogen in alleviating adverse effects of flooding on growth and yield of wheat in a sodic soil', *Field Crops Research*, vol. 35, no. 2, pp. 93-100.

Tan, W, Liu, J, Dai, T, Jing, Q, Cao, W & Jiang, D 2008, 'Alterations in photosynthesis and antioxidant enzyme activity in winter wheat subjected to post-anthesis water-logging', *Photosynthetica*, vol. 46, no. 1, pp. 21-27.

Taylor, AR & Bloom, AJ 1998, 'Ammonium, nitrate, and proton fluxes along the maize root', *Plant, Cell & Environment*, vol. 21, no. 12, pp. 1255-1263.

Tennant, D, Scholz, G, Dixon, J & Purdie, B 1992, 'Physical and chemical characteristics of duplex soils and their distribution in the south-west of Western Australia', *Animal Production Science*, vol. 32, no. 7, pp. 827-843.

Thomson, CJ, Colmer, TD, Watkin, ELJ & Greenway, H 1992, 'Tolerance of wheat (*Triticum aestivum* cvs Gamenya and Kite) and triticale (*Triticosecale* cv. Muir) to waterlogging', *New Phytologist*, vol. 120, no. 3, pp. 335-344.

Tilman, D, Fargione, J, Wolff, B, D'Antonio, C, Dobson, A, Howarth, R, Schindler, D, Schlesinger, WH, Simberloff, D & Swackhamer, D 2001, 'Forecasting agriculturally driven global environmental change', *Science*, vol. 292, no. 5515, pp. 281-284.

Trenkel, ME 1997, *Controlled-release and stabilized fertilizers in agriculture*, International fertilizer industry association (IFA), Paris, France.

Trenkel, ME 2010, *Slow-and controlled-release and stabilized fertilizers: an option for enhancing nutrient use efficiency in agriculture*, 2nd edn, International fertilizer industry association (IFA), Paris, France.

Trought, MCT & Drew, MC 1980, 'The development of waterlogging damage in young wheat plants in anaerobic solution cultures', *Journal of Experimental Botany*, vol. 31, no. 6, pp. 1573-1585.

Trought, MCT & Drew, MC 1981, 'Alleviation of injury to young wheat plants in anaerobic solution cultures in relation to the supply of nitrate and other inorganic nutrients', *Journal of Experimental Botany*, vol. 32, no. 3, pp. 509-522.

Tsay, Y-F, Chiu, C-C, Tsai, C-B, Ho, C-H & Hsu, P-K 2007, 'Nitrate transporters and peptide transporters', *FEBS Letters*, vol. 581, no. 12, pp. 2290-2300.

Turner, N 1992, 'Crop production on duplex soils: an introduction', *Animal Production Science*, vol. 32, no. 7, pp. 797-800.

Tylova-Munzarova, E, Lorenzen, B, Brix, H & Votrubova, O 2005, 'The effects of NH_4^+ and NO_3^- on growth, resource allocation and nitrogen uptake kinetics of *Phragmites australis* and *Glyceria maxima*', *Aquatic Botany*, vol. 81, no. 4, pp. 326-342.

van Herwaarden, A, Farquhar, G, Angus, J, Richards, R & Howe, G 1998, "Haying-off', the negative grain yield response of dryland wheat to nitrogen fertiliser. I. Biomass, grain yield, and water use', *Australian Journal of Agricultural Research*, vol. 49, no. 7, pp. 1067-1081.

Vartapetian, B & Jackson, M 1997, 'Plant adaptations to anaerobic stress', *Annals of Botany*, vol. 79, no. suppl 1, pp. 3-20.

Vidmar, JJ, Zhuo, D, Siddiqi, MY, Schjoerring, JK, Touraine, B & Glass, AD 2000, 'Regulation of high-affinity nitrate transporter genes and high-affinity nitrate influx by nitrogen pools in roots of barley', *Plant Physiology*, vol. 123, no. 1, pp. 307-318.

Voesenek, L, Colmer, T, Pierik, R, Millenaar, F & Peeters, A 2006, 'How plants cope with complete submergence', *New Phytologist*, vol. 170, no. 2, pp. 213-226.

von Wirén, N, Gazzarrini, S & Frommer, WB 1997, 'Regulation of mineral nitrogen uptake in plants', *Plant and Soil*, vol. 196, no. 2, pp. 191-199.

von Wirén, N, Gazzarrini, S, Gojont, A & Frommer, WB 2000, 'The molecular physiology of ammonium uptake and retrieval', *Current Opinion in Plant Biology*, vol. 3, no. 3, pp. 254-261.

Wang, F & Alva, A 1996, 'Leaching of nitrogen from slow-release urea sources in sandy soils', *Soil Science Society of America Journal*, vol. 60, no. 5, pp. 1454-1458.

Wang, MY, Glass, AD, Shaff, JE & Kochian, LV 1994, 'Ammonium uptake by rice roots (III. Electrophysiology)', *Plant Physiology*, vol. 104, no. 3, pp. 899-906.

Wang, MY, Siddiqi, MY, Ruth, TJ & Glass, AD 1993, 'Ammonium uptake by rice roots (II. Kinetics of $^{13}NH_4^+$ influx across the plasmalemma)', *Plant Physiology*, vol. 103, no. 4, pp. 1259-1267.

Wang, S, He, L & Li, Z 1996, 'A comparative study on the resistance of barley and wheat to waterlogging', *Acta Agronomica Sinica*, vol. 22.

Wang, XY, Gao, CB & Liu, ZY 2012, 'Effects of postponing N application on wheat grain yield, protein quality and fertilizer-N use efficiency in low-yield field in Jianghan plain', *Advance Journal of Food Science and Technology*, vol. 4, no. 6, pp. 357-361.

Watson, E, Lapins, P & Barron, R 1976, 'Effect of waterlogging on the growth, grain and straw yield of wheat, barley and oats', *Animal Production Science*, vol. 16, no. 78, pp. 114-122.

Weisenseel, MH, Dorn, A & Jaffe, LF 1979, 'Natural H^+ currents traverse growing roots and root hairs of barley (*Hordeum vulgare* L.)', *Plant Physiology*, vol. 64, no. 3, pp. 512-518.

Wenger, L 2010, 'Oxygen transport in waterlogged plants', in S Shabala & S Mancuso (eds), *Waterlogging signalling and tolerance in plants* Springer-Verlag, Berlin, pp. 10-11.

Wenyu, Y, Mingrong, H, Yuanjun, W, Xiaoying, W, Bin, Z & Cuiping, W 2005, 'Effect of controlled-release urea combined application with urea on nitrogen utilization efficiency of winter wheat', *Plant Nutrition and Fertiliser Science*, vol. 11, no. 5, pp. 627-633.

Wirth, J, Chopin, F, Santoni, V, Viennois, G, Tillard, P, Krapp, A, Lejay, L, Daniel-Vedele, F & Gojon, A 2007, 'Regulation of root nitrate uptake at the NRT2. 1 protein level in *Arabidopsis thaliana*', *Journal of Biological Chemistry*, vol. 282, no. 32, pp. 23541-23552.

Wolt, JD 1994, *Soil solution chemistry: applications to environmental science and agriculture*, Wiley, New York.

Wortmann, CS, Tarkalson, DD, Shapiro, CA, Dobermann, AR, Ferguson, RB, Hergert, GW & Walters, D 2011, 'Nitrogen use efficiency of irrigated corn for three cropping systems in Nebraska', *Agronomy Journal*, vol. 103, no. 1, pp. 76-84.

Wuest, S & Cassman, K 1992a, 'Fertilizer-nitrogen use efficiency of irrigated wheat: I. Uptake efficiency of preplant versus late-season application', *Agronomy Journal*, vol. 84, no. 4, pp. 682-688.

Wuest, S & Cassman, K 1992b, 'Fertilizer-nitrogen use efficiency of irrigated wheat: II. Partitioning efficiency of preplant versus late-season application', *Agronomy Journal*, vol. 84, no. 4, pp. 689-694.

Xu, G, Fan, X & Miller, AJ 2012, 'Plant nitrogen assimilation and use efficiency', *Annual Review of Plant Biology*, vol. 63, pp. 153-182.

Yaduvanshi, NPS, Setter, TL, Sharma, SK, Singh, KN & Kulshreshtha, N 2012, 'Influence of waterlogging on yield of wheat (*Triticum aestivum*), redox potentials, and concentrations of microelements in different soils in India and Australia', *Soil Research*, vol. 50, no. 6, pp. 489-499.

Yan, M, Fan, X, Feng, H, Miller, AJ, Shen, Q & Xu, G 2011, 'Rice OsNAR2. 1 interacts with OsNRT2. 1, OsNRT2. 2 and OsNRT2. 3a nitrate transporters to provide uptake over high and low concentration ranges', *Plant, Cell & Environment*, vol. 34, no. 8, pp. 1360-1372.

Youngdahl, L, Pacheco, R, Street, J & Vlek, P 1982, 'The kinetics of ammonium and nitrate uptake by young rice plants', *Plant and Soil*, vol. 69, no. 2, pp. 225-232.

Zadoks, J, Chang, T & Konzak, C 1974, 'A decimal code for the growth stages of cereals', *Weed Research*, vol. 14, no. 6, pp. 415-421.

Zeng, F, Konnerup, D, Shabala, L, Zhou, M, Colmer, TD, Zhang, G & Shabala, S 2014, 'Linking oxygen availability with membrane potential maintenance and K^+ retention of barley roots: implications for waterlogging stress tolerance', *Plant, Cell & Environment*, vol. 37, no. 10, pp. 2325-2338.

Zhang, H, Turner, N, Poole, M & Simpson, N 2006, 'Crop production in the high rainfall zones of southern Australia-potential, constraints and opportunities', *Animal Production Science*, vol. 46, no. 8, pp. 1035-1049.

Zhang, H, Turner, NC & Poole, ML 2004, 'Yield of wheat and canola in the high rainfall zone of south-western Australia in years with and without a transient perched water table', *Crop and Pasture Science*, vol. 55, no. 4, pp. 461-470.

Zhang, H, Turner, NC, Poole, ML & Asseng, S 2007, 'High ear number is key to achieving high wheat yields in the high-rainfall zone of south-western Australia', *Australian Journal of Agricultural Research*, vol. 58, no. 1, pp. 21-27.

Zhou, M 2010, 'Improvement of plant waterlogging tolerance', in S Shabala & S Mancuso (eds), *Waterlogging signalling and tolerance in plants*, Springer-Verlag, Berlin, pp. 267-285.